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1992

Center for Biologics Evaluation and
Research
Division of Biochemistry and Biophysics

Annual report
1991-1992



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DIVISION OF BIOCHEMISTRY AND BIOPHYSICS

Annual Report

October 1, 1991 through September 30, 1992

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SUMMARY

Summary by Director

General Activities of the Division of Biochemistry and Biophysics Related to FDA's Objectives and Responsibilities

The Division of Biochemistry and Biophysics was established in 1979 to support the mission of the Center for Biologics Evaluation and Research, FDA, through scientific research and its application to regulatory activities. It has placed a strong emphasis on interdisciplinary studies in basic sciences in the broad areas of Biochemistry, Biophysics, Developmental Biology, Molecular Biology, Cell Biology, Pharmacology, and Immunology. The Division has encouraged a balanced convergence of basic scientific research and regulatory activities. Many regulatory insights have been derived from our basic research activities, and in turn, many of our scientific contributions have had their roots in regulatory activities.

The research and regulatory activities of the Division have been summarized each year, without interruption since its inception, in the Division's Annual Report. We have always been and are proud to have been an organization that has always sought to support qualified individuals in basic research that has the intrinsic value even in areas that were not apparently related to our regulatory mission. We maintained the conviction that regulatory work should be based on sound scientific judgment, and that scientists who are dedicated to quality research and possesses good common sense could provide the best regulatory input. Indeed, by offering opportunities and facilities for laboratory research, we have been able to recruit and retain an outstanding group of scientists who have given vital support to the regulatory mission of CBER. Over the years, the Division has hosted over 160 scientific staff members and has benefitted from the participation of 50 Guest Workers. (Table I)

Staff in the DBB have contributed significantly to the regulatory activities of the Center. The Division was primarily responsible for the preparation of two "Points to Consider" documents, "Points to Consider in the Production and Testing of New Drugs and Biologics Produced by Recombinant DNA Technology, (1985)", and "Points to Consider in Somatic Cell Therapy and Gene Therapy, (1991)." The number of INDs reviewed by the Division staff has steadily increased each year, and in FY92 has exceeded 900 (Table II), covering practically all areas of products reviewed by the Center.

DBB scientists have established one of the best centers for the study of macromolecular structures and cellular structures, including pharmaceutical proteins, polysaccharide vaccines, oligonucleotides, membrane organization, and cellular structures. With the support of the Center Directors over the last two decades, the Division has assembled truly state-of-the-art technologies in biochemistry, biophysics, cell biology, and molecular immunology. Recognizing that the problems of biologics are often multifaceted and complex and that it would be impossible to maintain specific staff experts on every product and on every procedure, we have taken the approach of having a group of basic research scientists at the cutting-edge of relevant disciplines, who are broad enough to review such protocols and stay current at a time of explosive development in the field and in the literature. At the same time, the Division has continually evaluated new areas and new direction of research and has sought to maintain the flexibility to support research in promising frontiers of science that are

relevant to its mission. The increasingly important roles that the Division staff have evolved to play in the research and regulation of Somatic Cell/Gene Therapy and Monoclonal Antibody products are prime examples. DBB staff have chaired (and additional staff members have served on) licensing committees for three monoclonal antibody products, and have prepared a document establishing criteria for evaluation of tumor imaging agents. Staff members have conducted pre-license inspections for the product nearing approval, and have prepared a Summary Basis of Approval and Product Insert.

In somatic cell and gene therapy, activity has been in the IND area, with an explosive growth of a field that basically did not exist, to a field with a burgeoning number of applications. While the NIH-RAC also reviews gene therapy proposals, they have stated publicly that they rely upon CBER to handle issues of product manufacture and safety, and thus ultimately to protect the public in this volatile area.

The establishment of a Developmental Biology program some years ago is yet another good example. This program, which started with two senior scientists, one each in the Molecular Pharmacology and Cellular Biology Laboratories, has gained substantial momentum and strength in the last two years and will soon share major responsibility, within the somatic cell/gene therapy program, for the regulation of novel regeneration factors, an emerging area of importance in biologics.

Likewise, the parasitology program initiated several years ago with a single FTE and still essentially a one-man operation, is beginning to bear fruit. Understanding of the genetics of both the hosts and the parasites will ultimately help to identify new chemotherapeutic and vaccine targets. During the last two years, three of the heat-shock protein genes have been isolated and fully characterized from Plasmodium falciparum. These genes will be tested as vaccine candidates for malaria. Meanwhile, a number of INDs related to malaria vaccines have arrived at CBER for review and evaluation.

DBB staff also served a valuable role in FDA when silicone breast implants became an important regulatory issue. CDRH and the Commissioner's office sought expertise on autoimmunity to help them, and identified an authority in the field on the DBB staff. DBB involvement included providing testimony on general clinical and epidemiologic aspects of connective tissue diseases to an Advisory Panel meeting on silicone breast implants in February 1992, and participation in a task force on the subject.

As the Director of DBB, I have had the pleasure and satisfaction of seeing the initiation and growth of these more recent projects. Needless to say, I am equally proud and deeply gratified to witness the continuous growth and high productivity of the Biochemistry, Biophysics, Chemical Biology, and Analytical Chemistry programs; the programs that served as the original basis for the establishment of DBB.

Computer technology and recombinant DNA technology have ushered in a new era of science and an impressive new breed of biological products. This new wave of biological products has necessitated a re-assessment of the historical concerns over product purity, safety, potency, and efficacy. The establishment of a new

Division of Biochemistry and Biophysics in 1979 by the then Director of the Bureau of Biologics, was a response in anticipation to this challenge. Over the years, DBB has contributed a significant share to keeping CBER abreast with the cutting-edge science and in fulfilling many of its regulatory mission.

DBB scientists have served on numerous product licensing committees and participated in licensing inspections. The products being considered for licensing include monoclonal antibodies, various erythropoietin applications, polysaccharide vaccines, TPA, factor VIII, histamines, allergy patch tests, and AIDS and hepatitis test kits. These activities have enabled DBB staff members to develop diverse types of expertise that makes them sought after for their participation at regulatory meetings and on committees.

The basic principle and criteria by which the Division used to recruit its scientific staff are scientific excellence and personal integrity. Scientists who joined the Division initially found their rewards in pursuing their independent research with great enthusiasm and dedication. The Division has endeavored to support and encourage their pursuit of science. Almost always, following the establishment of a niche within the organization, the newly arrived scientist would seek an opportunity to participate in the regulatory mission of the Center. Most often the best regulatory input is generated by scientists who are performing quality scientific research.

As this Annual Report is being prepared, CBER is undergoing a comprehensive reorganization which will result in the termination of the existence of DBB. The staff of DBB will be reassigned to newly created Offices and Divisions. In the new structure, "former" DBB staff will continue to play significant roles, as Associate Director for Research, and Directors of newly created Divisions, and Chiefs of Laboratories. Their success in their new missions will bring honor to their old Division of Biochemistry and Biophysics. We look forward to their success and wish every one of them good luck. The Division also takes pride in having recruited the current Director of the Center as a member of the class of 1981.

Returning now to the Annual Report, in FY92 the Division staff reviewed 941 IND submissions and about 3000 protocol reviews in areas including cytokines, blood products, monoclonal antibodies, bacterial products, viral products, cellular products, and AIDS related products (Table II). The INDs included a variety of unusual products (see the list of miscellaneous IND's, footnote to Table II). The interdisciplinary nature of the DBB staff was particularly valuable in regulating them. Two thousand nine hundred and ninety nine chemical analyses were performed in support of over 40 product licenses and protocol reviews. The Division provides special resources for the physico-chemical characterizations of bio-pharmaceuticals including analyses of glycoproteins, vaccines, adjuvants, and polysaccharides.

The research activities pursued during the past year by the Division scientists are summarized in the individual annual reports. The work covers a diversified area ranging from transcriptional controls of genes, cloning and expression of the large surface antigen of Hepatitis B virus, the heat-shock proteins of the malaria parasite (Plasmodium falciparum), localization of the adenylyl-cyclase and glucose transporter in rat brain and the analyses of the B cell

differentiation pathway in transgenic mice. The Division has been organized to encompass a diversified field of biological investigators and structured to facilitate close interactions among its scientists. The significant research and regulatory accomplishments in which the staff of the Division participated as principal investigators or as collaborators are highlighted below.

- * Using B cell lineage tumors, arising in mice transgenic for oncogene expression, for the analyses of the B cell differentiation pathway, four stages in pro-B and pro-B cell development were determined. (Bauer)
- * A major neutralizing epitope of HIV has been mapped to position 341-511 of the envelope glycoprotein gp 120. This epitope is conserved among widely divergent HIV isolates and so may be a more suitable target for vaccine development. (Berkower and Co-workers)
- * Antibody responses to the rCD4 and cell-surface expressed CD4 preferentially recognized different epitopes, a finding that may have implications for development of vaccines including AIDS vaccines. (Epstein and Co-workers)
- * Clinical, serological, and immunogenetic features have been analyzed in myositis patients with and without silicone implants, and differences were detected. (Miller and Co-workers)
- * Molecular dynamics simulations of biologically active lipid bilayer has provided a detailed and accurate atomic level description of the membrane which will be useful for testing various theories, and further studying of the interaction of drugs and proteins with membranes. (Pastor and Co-workers)
- * Nine cis-acting sequences that bind 6 different trans-acting proteins were identified in the human C-Reactive protein gene. This has led to the discovery of a novel repressor for the CRP gene. (Goldman and Li)
- * Studies with the Xenopus CRP gene have shed light on the evolution of the function of CRP, e.g. immunoglobulin-like activity in the host defense mechanism. (Lin and Liu)
- * The large surface antigen of Hepatitis B virus has been cloned and expressed in the baculovirus. This study will establish biological equivalency of the large surface antigen as well as help to elucidate the viral receptor of the host cells. (DeVries)
- * Two 60 KDa proteins, Ro/SS-A and calreticulin have been found to bind to cis-acting sequences in the 5' and 3' ends of the rubella viral genome, respectively. Calreticulin is involved in the regulation of the viral genomic mRNA, the initial step in viral gene expression. (Nakhasi and Co-workers)
- * Three genes which undergo differential expression during Leishmanial

development have been isolated and characterized. The importance of these genes in the virulence of this parasite is being determined. (Nakhasi and Co-workers)

- * Three heat-shock proteins - hsp 90, hsp 60, and GRP 78 - were cloned from Plasmodium falciparum, the deadly strain of malaria. The proteins coded by those three genes will be tested as vaccine candidates for malaria. (Syn and Co-workers)
- * Xenopus homeobox genes involved in neural induction and regionalization of the neural plate and mesoderm were identified. (Jamrich and Co-workers)
- * A C-terminal domain of the P-glycoprotein multidrug resistance protein involved in drug binding has been identified. (Seamon and Co-workers)
- * The adenylyl cyclase and glucose transporter has been localized in the rat brain. (Seamon and co-workers)
- * An in-gel enzymatic digestion procedure for proteins has been developed for microsequencing of separated proteins. (Moos)
- * A facile and reliable procedure for the conjugation of large molecular weight polyethylene glycol macromolecules to the 3'-terminus of oligonucleotides has been developed for the study of cellular uptake to evaluate their efficacy in inhibiting translation of mRNA. (Beaucage and co-workers)
- * A Summary Basis of Approval (SBA) and product insert for a product reviewed under DBB leadership have been prepared and readied for approval. Pre-license inspections of the two relevant sites were performed.
- * The insertional specificity of a Drosophila transposable element has been altered by gene cloning and insertion. (Kassis and co-workers)
- * A flow facility network and prototype FDA Plot graphic package have been developed to expedite the quality control of monoclonal antibody products. (Noguchi and co-workers)
- * A method for identifying post-translational modification at the carboxy-terminal of polypeptides has been developed to facilitate the isolation and characterization of bioactive peptides. (Fraser and co-workers)
- * The theory of reversed DEPT and reverse INEPT NMR experiments, useful for the study of protein dynamics, has been developed. (Bull)
- * The effects of solvation on the aspartyl protease from HIV-1 has been investigated. The overall structure of the solvated monomer

was shown to be similar to the crystal structure. (Venable)

- * Various abasic oligonucleotide phosphorothioates were synthesized and evaluated for their ability to inhibit the replication of the HIV-1 virus in a de novo infection assay. (Egan and co-workers)
- * DBB staff have participated in more than a dozen Advisory Committee meetings.
- * DBB staff spoke at numerous conferences on regulatory matters.

The staff of the Division of Biochemistry and Biophysics continues to maintain state-of-the-art technology including microanalysis and sequencing of proteins, oligonucleotide synthesis, flow cytometry, and instrumentation including NMR, and mass spectrometer. These facilities have often served as the focal point of collaboration with scientists on the NIH campus and other FDA scientists. The availability of in-house expertise in these areas of high technology has provided the Center for Biologics Evaluation and Research with an excellent reputation among scientists in the academic community and industry.

Members of the staff served on editorial boards and reviewed manuscripts for The Journal of Biological Chemistry, Biochemistry, Journal of Immunology, Proceedings of the National Academy of Sciences, J. Analytical Biochemistry, J. Carbohydrate Chemistry, J. Medicinal Chemistry, Analytical Chemistry, J. Protein Chemistry, J. Interferon Research, Science, Cytometry, Cancer Research, J. National Cancer Institute, J. of the Association of Official Analytical Chemists, Molecular Pharmacology, J. American Chemical Society, Journal of Clinical Investigation, Arthritis and Rheumatism, J. Magnetic Resonance, J. Virology, Cell, EMBO J., Clinical Exp. Immunol. and Clinical and Experimental Rheumatology.

Many have been invited speakers at a number of national and international meetings, seminar speakers at various universities and educational institutions, as well as members of the National Science Foundation Grant Review Board, the NIH Grant Review Board, and project site-visits. Several staff members also serve in adjunct positions at local universities.

FY92 was a very productive year, the staff of the Division authored and coauthored 79 manuscripts, 49 manuscripts have appeared in journals, 30 manuscripts are in press.

Darrell T. Liu
Darrell T. Liu, Ph.D.

Sept 10, 1992
Date

Table I

Division of Biochemistry and Biophysics

1979 - 1992

Division Director: Darrell T. Liu, Ph.D.

Deputy Director: Philip D. Noguchi, M.D.

<u>NAME</u>	<u>POSITION-DBB</u>	<u>ARRIVED</u>	<u>LEFT</u>	<u>POSITION-PRESENT</u>
Ackerman, Samuel, M.D.	Senior Staff Fellow	82	83	Xoma
Anderson, Archie	Bio.Lab Tech.(Micro)	79	84-Retired	
Anderson, Lori	Clerk-Typist	82	84	
Abdul, Attallah, Ph.D.	Microbiologist	79	LWOP/86	Egypt/89
Baba, Elio	Visiting Scientist	88	91	Return to Brazil
Barron, Robert P.	Research Chemist	84	86	CDER, FDA
Bauer, Steven	Senior Staff Fellow	91		
Beaucage, Serge, Ph.D.	Staff Fellow	87		
Berkower, Ira, M.D.	Captain/PHS	82		
Bertin, Pablo, M.D.	Fogarty Fellow	89	92	Return to Chile
Biddle, Jane	Chemist	87	89	NIH
Bishop, John	Staff Fellow	92		
Boal, Jila, Ph.D.	Staff Fellow	88	92	U. of Rochester
Boehlert, Charles	Chemist	83	88	
Booth, Roberta	Biol. Aide	88	90	DV, CBER

Bower, Margaret	NRC Fellow	85	88	
Boykins, Robert	Biologist	79		
Browne, Walter	Biol. Lab Tech.	79	87-Retired	Deceased
Buko, Alexander	Staff Fellow	81	83	Industry
Bull, Thomas	Interagency Detail/ Research Chemist	85 86		
Byrd, R. Andrew	Research Chemist	83	92	PRI, Frederick, MD
Callahan, Lawrence	Staff Fellow	86	88	Am. Type Culture Collection
Cao, Xiqiang	Fogarty Fellow	88	92	NIH
Carter, Patricia	Biologist	79		
Caruso, Ellen	Editorial Asst.	79		
Chan, Sunney	Fogarty Scholar	86	87	Professor, Cal. Tech.
Cheng, Sheau-Mei	Staff Fellow	82	86	Industry
Chopra, Neeraj	Biological Aid	90	92	Medical School
Cislo, Teresa	Research Chemist	87	90	Industry
Clayton, Rebecca	NRC Fellow	89	91	U. of Maryland
Cosenza-Murphy, D.	Biologist	86		
Cotterman, Melissa	Microbiologist	87	89	NIH
Cunningham, Robert	Biologist	81	88	Walter Reed/AFI of Pathology

Daitch, Charles	Stay-In-School	87	89
DeIGrosso, Alfred	Chemist	79	
Desai, Indira	Off. Automation Asst.	87	
DeVries, Yuan	Research Chemist	87	
Dias, Consuelo	Vis. Fel./Fog. Fel	90	
Dickens, Lillian	NRC Fellow	87	90 NIH
Dietrichson, Lenetta	Clerk-Typist	88	88 NIH
DiNola, Liliana	Fogarty Fellow	88	90 Industry
Dirksen, Marie-Luise	Biologist	89	
Egan, William	Research Chemist	79	Chief, Lab Biophys.
Epstein, Suzanne	S. Fel./Res. Chem.	84/86	Chief, Lab Mol. Imm.
Etz, Nora	Chemist	79	
Ewell, John	Biologist	79	
Fang, Florence	Research Chemist	83	87 CDER
Ford, Leonard	Research Chemist	84	85
Fraser, Blair	Research Chemist	79	Chief, Lab Chem. Biol.
Frugoni, Patrizia	FDA Visit. Scientist	87	88 Return to Italy
Fujii, Nobutaka	FDA Visit. Scientist	84	86 Prof. Kyoto Univ., Japan
Gallo, Kathleen	Biol. Lab Tech.	82	85

Gates, Frederick	S. Fellow/Res. Chemist	81/83	87	Genetics Inst.
Goldman, Neil	S. Fel/Res. Chemist	82/87		Chief, Lab Biochem.
Grantham, Vicki	Secretary	86	89	NIH
Greenblatt, Jay	Staff Fellow	79	82	NCI
Grim, Mary	Chemist	79	84	EPA
Gurley, Rebecca	Biologist	91		
Hardy, Barry	NRC Fellow	90		
Hayden, Patrick	Bio. Lab Tech.	82	84	
Hoffman, Thomas	Surgeon, PHS	82	83	Chief, Lab Cel Biol, DH, CBER
Honemond, Crystal	Clerk-Typist	81	83	NIH
Hoskins, Joel	Biologist	85	87	
Iyer, Radhakrishnan	FDA Visit. Scientist	87	91	Return to India
Jamrich, Milan	Staff Fellow	88		
Jennings, Mary	Bio. Lab Tech.	82	83	
Jin, Yide	Fogarty Fellow	84	86	Univ. of Texas
Johnson, Joyce		79		Deceased
Jones, Karen	Microbiologist	82	89	DCB, CBER
Kassis, Judith	Staff Fellow	86		
Kindt, Rachel	Bio. Lab Aid	86	88	MIT Graduate Student

Koga, Masakazu	Fogarty Fellow	89	91	Return to Japan
Laurenza, Antonio	FDA Visit. Scientist	86	88	Industry
Lee, Carol	Secretary	79	89-Retired	
Lei, Ke-jien	Fogarty Fellow	81	84	NIH
Lewis, Kathy	Secretary	89		
Liang, Shu-Mei	Staff Fellow	81	86	Industry
Lim, Tang-Kuan	Fogarty Fellow	85	87	
Lindner, Wolfgang	FDA Visit. Scientist	85	88	Return to Poland
Lin, Leewen	Staff Fellow	89		
Li, Shi-Peng	Fog.Fel./Vis.Scient.	84/88		
Liu, Darrell	Research Chemist	79		Dir., DBB
Liu, Teresa	Chemist	82		
Lo, Chia-Yun	Biologist	89		
Lo, Shan-Shan	Fogarty Fellow	81	84	Industry
Marti, Gerald	Commander, PHS	87		
Matis, Louis	Staff Fellow	86	89	Frederick
May, Joan	Chemist	79		Chief, Lab Anal. Chem.
McDermott, Timothy	Stay-in-School	85	87	
McGrath, Philip	Biologist	79	91-Retired	

McHugh-Sutkowski, E.	NRC/Staff Fellow	88/92	
Medda, Sukumar	Staff Fellow	91	
Miller, Andra	NRC Fellow	91	
Miller, Frederick	Commander/PHS	90	
Minetti, Conceicao	Fogarty Fellow	89	
Mirsadeghi, Seid	NRC Fellow	86	88 Industry
Misplon, Julia	Microbiologist	85	
Missall, Kathy	Secretary	85	86
Moos, Malcolm	Staff Fellow	85	
Morris, Diane	Research Chemist	83	
Muller, Jacqueline	Senior Scientist/PHS	81	88 DV, CBER
Nakhasi, Hira	S.Fellow/Res.Chemist	83/88	
Nascimento, Evaldo	Fogarty Fellow	86	89 Professor, Brazil
Nelson, Carol	Staff Fellow	85	87
Nguyen, Nga	S.Fellow/Res.Chemist	81/82	90 DCB, CBER
Noguchi, Philip	Captain/PHS	79	Deputy Director, DBB
O'Hanlon, Terrance	Staff Fellow	92	
O'Leary, Timothy	Staff Fellow	85	87 Dir, Cell. Pathology, AFIP
Pastor, Richard	S.Fellow/Res.Chemist	83/89	

	Fogarty Scholar in	91/92	91/92	Prof. U. of Cambridge, U.K.
Perham, Richard	Residence			
Phillips, Lawrence	Research Chemist	81	91	NIH
Pike, Sandra	Microbiologist	83	89	DCB, CBER
Prasad, Ganesh	Fogarty Fellow	85	87	Return to India
Progar, Joseph	Chemist	79		
Protenzone, Rudolph	Research Chemist	82	83	
Reeves, James	S.Fellow/Res.Off-PHS	87/89		
Reeves, Patricia	Biologist	90		
Regan, Judith	Chemist	84		
Rellahan, Barbara	NRC Fellow	88	89	NIH
Remeta, David	Staff Fellow	88	90	Resigned
Resto-Ruiz, Sandra	Microbiologist	89	91	Resigned
Richardson, Barbara	Bio. Lab Tech.	85		
Ridge, Jeanette	Biologist	83	88	DPQC, CBER
Ritter, Alice	Staff Fellow	91		
Robbins, Joan	Research Chemist	79		
Robey, Frank	Research Chemist	79	88	NIH
Ronald, Christy	Chemist	88	91	Industry

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Rudikoff, Eva	Biologist	92		
Samson, Carrie	Chemist	83	88	Industry
Schaeffer, Valentine	Research Chemist	84		
Sadegh-Nasseri, S.	Staff Fellow	88	90	NIH
Schwarzkopf, Laura	Stay-in-School	84	84	
Seamon, Kenneth	S.Fellow/Res.Chemist	83/86		Assoc. Dir. Research, CBER
Sensabaugh, Suzanne	Biologist	89		
Shinosuka, Kazuo	Fogarty Fellow	84	87	Return to Japan
Shueh, Lisa	Bio. Lab Aid	86	88	Industry
Singh, Nishi	Fogarty Fellow	90		
Stec, Wojciech	Visiting Scientist	82	83	Professor, Poland
	Fogarty Scholar	92		
Stevens, James	Staff Fellow	82	85	Industry
Summers, Michael	Staff Fellow	83	87	Univ. Maryland
Swartz, Laura	Chemist	89		
Syin, Chiang	Staff Fellow	88		
Tanner, Jerome	Staff Fellow	87	89	DCB, CBER
Thakker, Dhiren	Research Chemist	84	89	Industry
Thomas, Desiree	Stay-in-School	84	90	U. PA Veterinary Sch.

Tosato, Giovanna	Med.S.Fel/Med.Off.	83/85	89	Chief, Lab Mol Imm, DCB/CBER
Unger, Sue	Secretary	89		
Uznanski, Bogdan	FDA Visit. Scientist	87	90	Return to Poland
VanSickle, Page	Biologist	88	90	VA Tech, Veterinary Sch.
Venable, Richard	Chemist	84		
Walling, MaryJane	Microbiologist	86	89	OBPR, CBER
Wang, Ning	Fogarty Fellow	84	86	Sch Public Health, Harvard
Wang, Zhizhen	FDA Visit. Scientist	87	91	Canada
Washington, Glennelle	Biologist	79		
Wheeler, Roscoe	Phys.Science Tech.	79		
Williams, Garth	Bio. Lab Tech.	83	84	
Wyne, Nancy	Secretary	79	89-Retired	
Yajima, Haruaki	Fogarty Scholar in Residence	79/80	79/80	Professor, Kyoto Univ. Japan
Zhang, Yao-Shi	FDA Visit. Scientist	84	87	
Zhang, Yuhong	Fogarty Fellow	90		
Zheng, Dexian	Fogarty Fellow	85	88	Return to China
Zon, Gerald	Research Chemist	81	87	Industry
Zoon, Kathryn	S.Fellow/Res.Chemist	81/82	83	Director, CBER

ZurNedden, Dorothy Bio.LabTech/Microbio 81 83 Spec. Asst., to Dir. CBER

<u>GUEST WORKERS</u>	<u>LABORATORY</u>	<u>ARRIVE</u>	<u>LEFT</u>	<u>PRESENT LOCATION</u>
Akagi, Kenichi	Biochemistry	86	88	Return to Japan
Awret, Uziel		82/83	82/83	
Ayoubi, Nasib		85/86	86/87	
Baba, Julia	Biochemistry	88/89	90/91	Return to Brazil
Badr, Ahmed	Grad. Worker	82/83	82/83	
Beydoun, Rafic		85/86	85/86	
Boal, Jila	Biophysics	85/86	87/88	Staff Fellow-88/89
Boyd, Victoria		84/85	84/85	
Bristol, James	Grad. Student	82/83	82/83	
Bull, Thomas	Biophysics	83/84	84/85	Regular GS Employee 86
Carson, Frederick		87/88	90/91	
Chun, Shun-Wen		85/86	85/86	
Enz, Lisa		91/92	91/92	
Fry, Alicia		87/88	88/89	Resident, Johns Hopkins Hosp.

Ghatak, Sudip	85/86	85/86	
Hakim, Asaad	83/84	84/85	
Hammadeh, Rasheed	85/86	85/86	
Hassan, Chaffar	83/84	84/85	
Helbaoui, Amer	85/86	85/86	
Hornicek, Francis	82/83	82/83	DCB, CBER
Hu, Ren-qiou	82/83	82/83	
Ibrahim, Mohamed	83/84	84/85	
Kang, Sangu	92	92	
Knop, Richard	83/84	84/85	
Larsen, Gerald	83/84	84/85	U. of Illinois, Med.Sch.
Laurenzo, Antonio	85/86	85/86	FDA Vis. Scientist 86-88
Love, Lori A.	91		CFSAN, FDA
Ludeman, Susan	85/86	88/89	Johns Hopkins University
Masheshwari, Radha	82/83	82/83	
Mesiona, Winonna	91	91	MARC Student
Metwalli, Ahmed	83/84	85/86	
Meyers, Jeff	85/86	85/86	
Minetti, Conceicao	88	89	Fogarty Fellow-89
			Biochemistry

Mishra, Savita	92	92	
Morad, Ammar	85/86	85/86	
Noel, Marianne	85/86	85/86	
Nunes, Paulo	84	85	Return to Brazil
Porello, Valery	85/86	86/87	
Ridge, Jeanette	82/83	82/83	GS Biologist-83/84
Sacks, William	82/83	82/83	
Sakmar, Thomas	79/80	79/80	Rockefeller U., Asst.Prof.
Shafey, Omar	91	91	Arthritis Found. Fellow
Shao, Kai-Liu	84/85	85/86	Industry
Siddiqui, Hammida	85	86	
Stalder, Anne	84/85	84/85	
Stec, Bozenna	82/83	82/83	Return to Poland
Sutkowski, Elizabeth	87/88	87/88	NRC Fellow-88/89
Suzuki, Akira	83	86	Return to Japan
Syin, Chiang	82	86	Staff Fellow-88
Thourer, James	89	89	
Tran, Tin	91/92	91/92	FAES Fellow (92)
Turujam, Saleh	87/88	89/90	

Vaillancourt, Richard	Cel. & Mol. Bio.	83/84	84/85	
Wang, Kenneth		92	92	Howard Hughes Intern
Whiteley, Mary		89/90	90/91	Fogarty Fellow-92
Yamashita, Michio		84	85	Return to Japan
Yuan, Min		85/86	85/86	
Zapolo, Peter		92	92	
Zhang, Jia-Ming		82	82	Return to China, Div. Inst. Biologics

Table II
Division of Biochemistry and Biophysics

IND Review

September 1, 1991 - August 28, 1992

Month	Total	Cytk.	Blood	Mono.	Bact.	Vir.	AIDS	Misc.*	Somct
Sep.91	81	16	8	23	8	1	5	17	3
Oct.91	88	16	3	23	8	4	18	9	7
Nov.91	72	11	2	30	6	1	7	10	5
Dec.91	95	17	4	25	7	6	14	19	3
Jan.92	90	16	4	26	11	5	13	14	1
Feb.92	83	19	3	17	12	8	8	10	6
Mar.92	127	25	6	37	11	3	17	19	9
Apr.92	104	32	13	27	6	1	11	12	2
May 92	70	14	2	22	3	2	5	15	7
Jun.92	65	18	2	18	4	4	4	10	5
Jul.92	46	11	1	15	4	0	1	11	3
Aug.92	20	6	0	4	2		4	0	1
Total	941	197	52	265	83	36	105	145	55
Percent		21	6.0	28	9.0	4.0	11	15	6.0

*Misc. Allergen Patch Test 3, Allergic contact dermatitis, Allyl-8 oxyquanosine (adjuvant), Anti-mod modules, Antigen-S preparation, Autologous subcellular tumor vaccine, Autologous immune complex, Asparaginase + PEG, Bovine retinal s antigen (Uveitis), Cell culture equipment for TIL cells, Chromatographic resins, Cultured human myoblast, DEAE spherodex M/LS, DNase (DNase), DNA antiserum, DTG-DPG formulation in liposomes, Filtration products, GHK, Gly-His-Lys tripeptide copper chelate, Gly-L-His, L-Lys, Guanosine loxoribine, Immunoabsorbent A+B for human use, Immuther, Indium 111-DTPA-IgG, Liposomal malaria vaccine, Magnetic microspheres, Peptide-T, P. falciparum, Plasmodium falciparum malarial vaccine, Plasmodium vivax malarial vaccine, MDP analogue, MDP coupled autologous tumor cells vaccine, Oral S-AG preparation, Poly I.Poly C, Poly I.Poly C Cl2U, Porous spherical beads/dextran, Protease inhibitor, rDNase, QMA spherosil DMF, Tumor polysaccharidal substance, Soluble antigen from pooled ovarian carcinoma cell membranes + complete Freund's adjuvant, Soluble tumor antigen, T cell depletion device.

SUMMARY OF CURRENT RESEARCH FINDINGS

ANALYTICAL CHEMISTRY LABORATORY SUMMARY (1991-1992)

Personnel

Joan C. May, Ph.D. (Supervisory Chemist), Chief
Alfred Del Grosso, (Chemist)
Nora Etz, (Chemist)
Joseph Progar, (Chemist)
Laura Swartz, (Chemist)
Roscoe Wheeler (Physical Science Technician)

The Analytical Chemistry Branch of the Division of Biochemistry and Biophysics is responsible for research involving the quantitative, qualitative and/or structural identification of organic and inorganic chemical constituents and impurities in biological products. It is responsible for determining quantitatively the amount of preservatives, adjuvants, stabilizers, residual moisture, nitrogen, aluminum, mercury and other chemical constituents or contaminants in biological products for use in man which are subject to license and/or release by the Center for Biologics Evaluation and Research. It plans and conducts research to provide a broad theoretical basis for the application of analytical chemistry to the control of biological products. It develops new or improved test methods utilizing gas chromatography, liquid chromatography, atomic absorption spectrometry, UV-visible spectrophotometry, thermogravimetry, mass spectrometry, and other analytical methods. With regard to these responsibilities the following is a discussion of the work that has been accomplished this year.

Approximately 230 protocol chemical reviews are accomplished in our laboratory per month along with over 2999 chemical analyses (enumerated in Table 1 attached) performed per year in support of over 30 product licenses, amendments, and IND's or protocol reviews. Approximately 500 of these analyses for moisture, formaldehyde, aluminum, protein, thimerosal, phenol, glutaraldehyde and 2-phenoxyethanol have been done on vaccines and toxoids that are included in the National Vaccine Program. In addition, our group has been involved in reviewing chemical aspects of over 42 product license applications and amendments and IND's this year.

Dr. May has been involved in organizing an International Symposium on Biological Product Freeze-Drying and Formulation in conjunction with the International Association of Biological Standardization. The purpose of this symposium was to bring together new information on freeze-drying and formulation of vaccines and other biological

products including products resulting from recombinant and hybridoma methodologies. The meeting was held October 24-26, 1990 at the Masur Auditorium on the N.I.H. campus with approximately 500 people in attendance. The proceedings from the International Symposium on Biological Product Freeze-Drying and Formulation were published this year by the International Association of Biological Standardization as volume 74 in the series entitled Developments in Biological Standardization with Joan May and Fred Brown as Acting Editors. The paper by J.C. May, R.M. Wheeler, N. Etz, and A. Del Grosso entitled "Measurement of Final Container Residual Moisture in Freeze-Dried Biological Products" was also published in this volume.

Nora Etz will be presenting a paper at the 204th Meeting of the American Chemical Society in Washington, D.C. in August of 1992. The paper is entitled "Estimate of Protein Content in Certain Biological Products" by Nora Etz and Joan May. The protein assay is done to verify the specified amount of protein per dose. Assay results for the protein content of Haemophilus b Conjugate Vaccine and Hepatitis B Vaccine, Recombinant analyzed by the Bradford, o-phthaldehyde (OPA), ninhydrin, Lowry, micro-Kjeldahl and enzyme immunoassay (EIA) methods are compared. Protein value are determined for the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 927 (4%) Bovine Serum Albumin by several methods. Many of the standard methods for total protein determination when applied to biological products exhibit interferences from preservatives, adjuvants and other product constituents.

Dr. May was invited to present a paper at the 21st North American Thermal Analysis Society Conference in Atlanta, Georgia in September of 1992. The paper is "TG/MS Determination of Moisture in Allergen Patch Test Samples" by Joan C. May, Roscoe M. Wheeler, and Alfred V. Del Grosso. TG/MS has been applied to the analysis of the moisture content of Allergen Patch Test samples. The Allergen Patch Test is used to identify the specific chemical(s) responsible for allergic contact dermatitis. In one type of Allergen Patch Test the allergen is incorporated into a gel, which is formed into a paper-thin coating on a polyester sheet, dried, and cut into 0.81 cm² patches. The patches with different allergens are attached onto a tape and enclosed in a packet forming a standard test kit. This strip which may contain as many as twelve different allergens is taken from the packet and applied. The moisture content of the gel patch can be related to the stability of the allergen in the patch. Thermogravimetry/mass spectrometry (TG/MS) data were collected for Cain Mix, Thiuram Mix, Colophony and Balsam of Peru patches. TG/MS results can be used to differentiate among the types of materials which are components of the patches.

The paper entitled "Aluminum Content of Source Plasma and Sodium Citrate Anticoagulant" by Joan May, T.C. Rains, L.J. Yu and N. Etz has been published in Vox Sanguinis this year.

Work is continuing by Joseph Progar with the ICP and graphite furnace to lower the detection limit for aluminum in AHF and serum. Microwave digestion techniques are being explored to deal more effectively with

the digestion of AHF, The National Institute of Standards (NIST), formerly the National Bureau of Standards has completed the analysis of a reference preparation of 5% Albumin for aluminum by two different analytical techniques. This material is available as a control for assay work with aluminum in 5% Albumin and 25% Albumin as well as AHF. The reference material was filled at CBER. Aluminum is of concern since it has been linked to bone deterioration. High levels of aluminum have been determined in occasional lots of 25% Albumin. A manuscript describing this work is in preparation by Joseph Progar.

The Allergenic Products Manufacturers Association met in October of 1991. Dr. May presented the status of the perchloroethylene study. The National Institute of Standards and Technology (NIST), formerly the National Bureau of Standards, has assayed perchloroethylene allergenic extracts and pollens treated with perchloroethylene during purification steps. They have the capabilities to do capillary gas chromatography with nickel-63 electron capture detection and this type of gas chromatography interfaced to a mass spectrometer to perform confirmation work for both ultra trace and macro amounts of perchloroethylene. This analytical work began in 1988 in connection with discussion with the Allergenic Products Manufacturers Association in meeting with CBER and is continued into 1992. Perchloroethylene levels have been determined in several pollens and corresponding extracts by NIST. Related work with Drs. Charles Ris and Bill Farland of the EPA involves their performing a risk assessment for perchloroethylene in the allergenic extracts considering that perchloroethylene is a possible human carcinogen. This risk assessment has been completed this year. The Allergenic Products Manufacturers Association has also sponsored a perchloroethylene risk assessment. The risk assessment by the EPA will assist in interpreting the manufacturers results and also accumulating information concerning the risk assessment for this chemical at the levels determined by NIST in the pollens and the resulting allergenic extracts. Appropriate gas chromatographic equipment with purge and trap capability has been installed at CBER in the Analytical Chemistry Laboratory to do the assay for perchloroethylene. A statistically significant number of samples will be assayed for perchloroethylene in perchloroethylene treated pollens and respective allergenic extracts. This year Allergenic Products Manufacturers have provided information about the number of lots and types of lots of pollen treated with perchloroethylene. Dr. Horne and Dr. Rastogi in CBER's Division of Biostatistics and Epidemiology have analyzed the data and set the number of pollen samples and extracts that must be tested for perchloroethylene to provide a statistically significant sample size for the risk assessment. These sample will be provided to CBER by the Allergenic Product manufacturers.

The Allergenic Products Advisory Panel met in December of 1991. Al Del Grosso and Dr. May presented issues concerning perchloroethylene and the status of poison ivy extracts. Al Del Grosso discussed the issues involved in analyzing poison ivy extracts and the regulatory consequences for stability and potency. Dr. May summarized the work done by NIST in analyzing perchloroethylene treated pollen, blank

pollen, and allergenic extracts derived from perchloroethylene treated pollen. She also gave the status of the EPA risk analysis for perchloroethylene in pollens.

Dr. May is continuing as the Chairperson of the License Amendments for Allergen Patch Test as well as Positive Skin Test Control-Histamine. The Analytical Chemistry Laboratory assays these materials for potency. One manufacturer of Allergen Patch Test has submitted an amendment that has added approximately 12 more patches. Potency and stability concerns for these patches have been considerable. Development work on the assay for the low level of histamine (10 ppm) being licensed is also considerable.

New projects this year include undertaking the analysis of residual DNA in vaccines and biological products. We have purchased a Threshold Analyzer to evaluate residual DNA analysis by this procedure. We have undertaken a limited collaborative study to obtain residual DNA data comparing the hybridization method with the Threshold method for two different samples. Dr. James Robertson from National Institute for Biological Standards and Control in England and and Julian Peetermans from Smith Kline Beecham have provided materials that can be used as standards. We have also undertaken the analysis of ethyleneimine in IL-11. Ethyleneimine occurs as a residual of the use of polyethyleneimine in manufacture. Ethyleneimine is a potent carcinogen. We are also using the gas chromatographic procedure for the determination of polyribosyl-ribitol-phosphate (PRP) in Diphtheria Tetanus Acellular Pertussis Haemophilus Conjugate Vaccine. This assay has previously been done by Dr. Frasch's Laboratory.

Book Edited:

May, J.C. and Brown, F., Acting Editors for International Association of Biological Standardization, Developments in Biological Standardization, Vol. 74, Proceedings of International Symposium on Biological Products Freeze-Drying and Formulation, Bethesda, MD, 1990, Karger, Basel, 1991.

Published Manuscripts:

May, J.C., Wheeler, R.M., Etz, N., and Del Grosso, A.: Measurement of Final Container Residual Moisture in Freeze-Dried Biological Products. In: Developments in Biological Standardization, Proceedings International Symposium on Biological Product Freeze-Drying and Formulation, Bethesda, MD, 1990, Vol.74, pp.153-164, Karger, Basel, 1991.

May, J.C., Rains, T.C., Yu, L.J. and Etz, N.: Aluminum Content of Source Plasma and Sodium Citrate Anticoagulant, Vox Sanguinis, 62:65-69, 1992.

Published Abstracts:

Etz, N., and May, J.C.: Estimate of Protein Content in Certain Biological Products, 204th Meeting of the American Chemical Society, Washington, D.C., August, 1992.

May, J.C., Wheeler, R.M., and Del Grosso, A.: TG/MS Determination of Moisture in Allergen Patch Test Samples, 21st Meeting of the North American Thermal Analysis Society, Atlanta, Georgia, September, 1992.

ANALYTICAL CHEMISTRY LABORATORY Plans for 1992-1993

During 1990-1991 the Analytical Chemistry Laboratory plans to make progress in the development of new and improved analytical methodology for the analysis of preservatives, adjuvants, stabilizers, residual moisture and other constituents of injectable biological products and continue the analysis of complex organic molecules such as carba mix in Allergen Patch Test by high performance liquid chromatography, (HPLC), gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS) and thermogravimetry/mass spectrometry (TG/MS).

Major emphasis this coming year will be placed on:

(1) Continuing the development and improvement of facilities for metals analysis. Installation of an updated computer for the control of an atomic absorption spectrometer with Zeeman background correction was completed in the laboratory this year. A microwave digestion apparatus has also been installed. Plans are being made to install a class 100 clean hood. The primary purpose of this hood would be for improved sample handling procedures for aluminum analysis in albumin. Manufacturers are being encouraged to meet a maximum limit for aluminum content of 200 ppb.

(2) Continuing the development of alternate methodology for the determination of residual moisture in freeze-dried biological products. The TG/MS combination developed in our laboratory is providing us with information that is enabling us to differentiate between adsorbed water and water of decomposition found in freeze-dried biological products with complex thermograms such as Limulus Amebocyte Lysate. Improved coulometric Karl Fischer moisture analyzers are now available that have increased sensitivity. Many freeze-dried products are now being manufactured in single dose vials with very small amounts of freeze-dried material present. This new Karl Fischer instrumentation permits moisture analysis on a smaller number of vials and also increases the precision of analyses.

(3) Continuing the development of chromatographic capability. Computer programs and gas chromatographic equipment will be updated this coming year. A gas chromatograph with dedicated capillary column and a purge and trap capability has been purchased and installed this year. This has been obtained for work with perchloroethylene in allergenic extracts. Work will continue on a project involving the development and evaluation of silica-based metal-chelating stationary phases for high performance liquid chromatography.

(4) Establishing capability to assay for residual DNA in biotechnology products. The Threshold instruments has been purchased this year.

TABLE 1. A LISTING OF THE MAJORITY OF THE PRODUCTS, TESTS AND NUMBER OF TESTS PERFORMED IN THE ANALYTICAL CHEMISTRY LABORATORY IN 1991-1992.

<u>PRODUCT AND TEST PERFORMED</u>	<u>NUMBER OF TESTS</u>
Acellular Pertussis Vaccine with Diphtheria and Tetanus Toxoids	
aluminum	80
Formaldehyde	77
AIDS serum	
Moisture	1
Albumin	
Aluminum	123
Allergenicics	
Azide (latex)	25
Histamine (latex)	490
Glycerin	487
Phenol	136
Moisture-Vespids	98
Moisture-other	8
Tetrachloroethylene	22
Kjeldahl nitrogen	14
Ninhydrin protein (latex)	34
Allergen Patch test	
Balsam of Peru	8
Black Rubber Mix	36
Carba Mix	12
Cinnamaldehyde	25
Colophony	13
Nickel	17
p-phenylenediamine	8
Fragrance Mix	24
Mercaptobenzothiazole	16
Parabenz	6
p-tert-butyl-formaldehyde	14
Moisture	22
Thimerosal	16
Wool Alcohols	8
Formaldehyde	21

Anticoagulant	
Aluminum	59
Antihemophilic Factor	
Moisture	6
Aluminum	66
Polyethylene glycol (PEG)	4
Antihemophilic Factor (Recombinant)	
Moisture	5
Anti-inhibitor Coagulant Complex	
Moisture	6
Antithrombin III	
Moisture	3
BCG Vaccine	
Moisture	142
Botulinum Toxin	
Moisture	15
Cytomegalovirus Immune Globulin	
Moisture	9
Diphtheria and Tetanus Toxoids and Pertussis Vaccines Adsorbed	
Aluminum	4
Thimerosal	65
Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine, Adsorbed	
Thimerosal	24
Glutaraldehyde	11
2-phenoxyethanol	12
Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine, Adsorbed and Haemophilus b Conjugate Vaccine (Diphtheria Toxoid Conjugate) Combined	
Thimerosal	18
Polyribosyl-ribitol-phosphate (PRP)	58
DTP/Haemophilus b Conj. Vaccine	
Formaldehyde	13
Thimerosal	18

Factor VII	
Moisture	2
Factor VIII	
Moisture	4
Haemophilus type b Polysaccharide Conjugate	
Moisture	11
Hepatitis B Vaccine, Recombinant	
Aluminum	20
Influenza Virus Vaccine	
Formaldehyde	51
Thimerosal	36
Kjeldahl nitrogen	72
Interferon	
Moisture	19
Interleukin-2	
Moisture	37
Residual SDS	26
Intravenous Immune Globulin	
Moisture	3
Kjeldahl nitrogen	10
Japanese Encephalitis Vaccine	
Molecular Sizing	4
Formaldehyde	21
Lowry protein	113
Thimerosal	2
Moisture	3
Limulus Amebocyte Lysate	
Moisture	37
Lowry protein	14
Kjeldahl nitrogen	6

Rabies Vaccine	
Moisture	
Formaldehyde	10
Recombinant Human Granulocyte Macrophage Colony Stimulating Factor	
Moisture	4
Shigella	
Thimerosal	42
Streptokinase	
Moisture	4
Staphylococcus aureus	
Thimerosal	6
Tetanus Toxoid, Adsorbed	
Aluminum	26
Thimerosal	24
Urokinase	
Moisture	13

Biochemistry Laboratory Summary

Personnel:

Darrell T.-Y. Liu, Ph.D., Director, DBB
 Neil D. Goldman, Ph.D., Microbiologist, LBC, DBB
 Hira L. Nakhasi, Ph.D., Research Chemist, LBC, DBB
 Yuan L. DeVries, Ph.D., Research Chemist, LBC, DBB
 Chiang Syin, Ph.D., Staff Fellow, LBC, DBB
 Leewin Lin, Ph.D., Staff Fellow, LBC, DBB
 Gregory P. Pogue, Ph.D., Staff Fellow, LBC, DBB
 Shipeng Li, M.S., Visiting Scientist, LBC, DBB
 Conceicao Minetti, Ph.D., Visiting Fellow, LBC, DBB
 Nishi K. Singh, Ph.D., Visiting Fellow, LBC, DBB
 Consuela Dias, Ph.D., Visiting Fellow, LBC, DBB
 Robert Boykins, B.S., Biologist, LBC, DBB
 Teresa M.-Y. Liu, M.S., Chemist, LBC, DBB
 John Ewell, B.S., Biologist, LBC, DBB
 Patricia Reeves, B.S., Microbiologist, LBC, DBB

The theme of the research in the Biochemistry Laboratory is the study of the structure, function and regulation of macromolecules. The subject matters under study include: (1) acute phase proteins and their genes, (2) the role of host proteins in the replication of human pathogenic viruses, and (3) parasite antigens and their developmental regulation.

A recent approach to addressing diseases caused by genetic defects is the use of somatic cell gene therapy. Treatment of many of these diseases will require controlling the expression of the exogenously added gene so that the resulting product (e.g. a protein) is being produced in a therapeutically effective dose range, in the appropriate cell or tissue, and possibly in a temporal manner or in response to a specific biological response modifier. One of the major mechanisms of the regulation of gene expression is at the level of transcription. As a model system, we have been studying the cytokine regulation of the prototype acute phase reactant in the liver, C-reactive protein (CRP). S. Li and N. Goldman have shown that induction of CRP gene expression was mediated by two interleukin-6 (IL-6) responsive cis-acting elements (6RE) in the promoter region of the gene. The expression was further increased by two upstream constitutive enhancers. They have found that these two 6REs bound a constitutive and an IL-6 inducible trans-acting factor, NFIL-6. In addition, 7 other cis-acting elements which bound a total of 6 novel constitutive and inducible positive and negative trans-acting factors have been identified, including: HNF-1, HNF-3, and several Octamer-like factors. They have shown that the upstream 6RE was positively regulated while the downstream 6RE was negatively regulated. Further studies will be performed to identify and clone these novel repressors as well as determine the modifications which are responsible for their activation.

To further study the evolutionary origin and physiologic function of CRP, L. Lin and D. Liu have isolated and sequenced the cDNA for this protein from a Xenopus library. Using the cDNA clone, they have also identified the genomic structure of this gene. Comparison of CRP from the frog to that of other species indicated shared identity as high as 45% to human CRP. Higher

regional identity has led to a predicted location for the phosphorylcholine binding site. In addition, studies indicated that frog CRP was not induced by inflammation and was expressed in mature liver but not during the early stages of development.

Although there is a live virus vaccine to protect one from rubella virus infection, new cases still occur each year. The persistence of the virus in the unvaccinated population continues to place mothers in their first trimester at risk. The mechanisms involved in viral replication, mutation and teratogenesis are still unknown. H. Nakhasi, N. Singh, G. Pogue and D. Liu are focusing their studies to directly address the mechanism of rubella virus replication. They have identified the cis-acting RNA sequences at the 3' end of the viral genome and the negative-strand which appear to play important roles in viral replication. They have shown that these two elements bind both common as well as unique host proteins. They have identified two 60 kDa host proteins which bind to the stem-loop (SL) structures at both the 5' and 3' end of the genomic RNA. These two proteins appear to be Ro/SS-A and calreticulin. In vitro and in vivo translation of chimeric CAT RNA constructs containing the 5' and 3' SL structures indicated a cooperativity of these two elements for efficient translation. Antibody inhibition assays and deletions within the 5' SL identified Ro/SS-A as an important factor in translation of these chimeric RNAs. Recently, they have identified the protein which binds to the 3' genomic SL as calreticulin. The importance of this protein in the replication of the negative strand is being evaluated. The elucidation of the mechanism of viral replication of the negative strand is being evaluated. The elucidation of the mechanism of viral replication and the contribution of the host factors to this process is crucial not only to the understanding of the acute infection in man, the potential persistent infection which may ensue, and the teratogenic effect to the fetus; but it may also provide potential targets for new antiviral therapeutics as well as demarcate susceptible genomic regions for mutagenesis in order to produce potentially safer vaccine strains.

The WHO considers hepatitis B virus (HBV) infection amongst the top five most serious infections worldwide. Although vaccines are available, asymptomatic carriers are the biggest problem. Not only are they a source of new infection, but also they are reinfecting their own tissues and thus increasing the chance of HBV induced hepatocellular carcinoma. The HBV surface antigen (HBsAg) is the major envelope protein on the virion, yet little is known about the attachment of virus to hepatocytes. Recent evidence indicates that peptide segments aminoterminal to the small form of HBsAg, referred to as pre-S1 and pre-S2, may participate in the interaction of virus and host cell. Y. DeVries has prepared a HBV pre-S1-fusion protein in E. coli which can be cleaved to give rise to the PreS1 peptide alone. She has found that this peptide binds to the plasma membrane of liver cells. Y. DeVries and P. Reeves currently are producing the large (preS1+preS2+small) HBsAg in a baculovirus expression system. This full-length protein will be used to isolate and characterize the viral receptor(s) on hepatocytes.

Studies are also in progress to determine the primitive defense mechanisms which are utilized by an ancient species, Limulus polyphemus, to fight infections, in an attempt to extrapolate this for possible clinical use in man. C. Minetti, T. Liu, Y. DeVries and D. Liu have cloned two proteins from the horseshoe crab, a 12 kDa and a 55 kDa species. The 55 kDa protein,

referred to as limunectin, consists of a 45 amino acid domain repeated 10 times which has structural and functional similarities to adhesion molecules, e.g. vitronectin. This protein has both cell adhesion and agglutination properties. The 12 kDa protein is a basic protein which acts as an agglutinin and binds to Gram positive and negative bacteria, in particular, endotoxin. In addition, it has protease inhibitory activity. C. Dias and T. Liu are also studying the enzymes (e.g., serine proteases) and inhibitors (e.g., α 2-macroglobulin) which act in the clotting cascade. The function and utility of these proteins (e.g., in endotoxemia and blood-clotting deficiencies) will be considered.

Malaria is responsible for 100 million infections and at least 2 million deaths each year, worldwide. Presently, there is still no effective vaccine for this disease. C. Syin and N. Goldman are studying the genes involved in the stage differentiation, from asexual to sexual blood stage, of the malaria parasite as potential targets for drugs or vaccines. Recent studies of infectious diseases have implicated the importance of heat shock proteins (hsp) in pathogens (e.g., Mycobacteria) for development and survival in the host. They have identified and sequenced the cDNA and genomic clones for two heat shock proteins from Plasmodium falciparum: hsp90 which regulates protein folding and binding to transcription factors in the nucleus, and hsp60 which regulates protein folding and binding to transcription factors in the mitochondrion. In addition, they are cloning and sequencing a third hsp, GRP78, which regulates protein assembly and translocation in the endoplasmic reticulum. RNA analysis indicated that hsp90 transcription increased during gametogenesis. Understanding the molecular events in stage differentiation in humans, which is obligatory for the successful cycling of the malaria parasite, may lead to a way to interrupt the cycle, end infection or lessen the severity of disease (e.g., prevent neurovascular sequestering of infected red blood cells).

Leishmaniasis is a major parasitic disease in the world for which there still is no effective vaccine. Some U.S. troops (at least 30 reported cases) who served in the Persian Gulf have returned with active infection by Leishmania tropica. H. Nakhasi, M. H. Joshi and D. Dwyer have been studying the mechanism of differentiation of Leishmania from the promastigote form (found in the sandfly) to the amastigote form (found in the human macrophage). They are utilizing an in vitro culture system which mimics the changes in the parasite's environment as it moves from the insect vector to the phagolysosome of the macrophage (temperature, pH, CO₂ concentration). They have constructed cDNA libraries to each in vitro stage, and have been probing these libraries for RNAs which specifically were augmented in each of the stages. Three such clones have been identified and sequenced. The importance of the differential expression of these genes during the development of the parasite will be determined.

R. Boykins and J. Ewell continue to provide strong support for quality control of new recombinant products (e.g. AIDS-related peptides, viral and bacterial vaccines and cytokines). They also provide an integral part in the ongoing research in the Laboratory by providing necessary resources (e.g., synthetic peptides and oligonucleotides).

Publications

Liu T, Lin Y, Cislo T, Minetti CASA, Baba JMK, Liu T-Y. Limunectin: A phosphocholine binding protein from Limulus amebocytes with adhesion promoting properties, J Biol Chem 1991;266:14813-21.

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Liu T-Y. Deamidation: a source of microheterogeneity in pharmaceutical proteins, Trends in Biotechnology, 1992; in press.

Lin L, Liu T-Y. Isolation and characterization of Xenopus C-reactive protein cDNA and genomic DNA, J Biol Chem 1992; in press.

BIOPHYSICS LABORATORY SUMMARY

Personnel

William M. Egan, Ph. D., Research Chemist, Chief
Thomas E. Bull, Ph. D., Research Chemist
Richard W. Pastor, Ph.D., Research Chemist
Richard Venable, M. S., Chemist
Alice Ritter, Ph.D., Staff Fellow
Krystyna Lesiak, Ph. D., Staff Fellow
Alberto Gutierrez, Ph.D., Staff Fellow
Michelle Elam, Laboratory Technician
Yuhong Zhang, Ph.D., Fogarty Fellow
Barry Hardy, Ph.D., NRC Fellow
Terrence Henderson, Ph.D., NRC Fellow
Andrew Byrd, Ph.D., Guest Worker
Wojciech Stec, Ph.D., Guest Worker

Overview:

The research program of the Biophysics Laboratory is directed towards developing a characterization and understanding of the basic physical chemical properties of biological macromolecules (polysaccharides, proteins, lipids, and DNA) and the interaction of small molecules with these macromolecular systems. The principal research methodologies used by the laboratory include nuclear magnetic resonance (NMR) spectroscopy and theoretical calculations in conjunction with high-resolution computer graphics. Both of these rapidly changing and expanding fields present significant challenges to the laboratory to remain at the scientific forefront. During this past year, the 500 MHz NMR spectrometer was updated (funded the previous year); these replacements to the spectrometer provide an increased sensitivity along with the ability to carry out many new experiments (triple resonance, shaped pulses, etc.). The laboratory's computer facilities are in need of update and will be the main priority for the laboratory for the coming year.

There have been a number of changes in personnel this past year. Dr. Byrd has left to direct a program in protein structural studies at the NCI in Frederick. Dr. Jila Boal, a Staff Fellow with Dr. Egan, has left to take a position at the University of Rochester.

Dr. Krystyna Lesiak was hired into the laboratory and will be directing the laboratory's DNA facility; Ms. Michelle Elam, an undergraduate at Gaillardet College, was hired as a part-time laboratory technician and is working with Dr. Lesiak on the synthesis and purification of nucleic acids; Dr. Lesiak moved into the position vacated by Christy Ronald, who left last summer to take a position with Hewlett Packard.

Dr. Alberto Gutierrez has also joined the laboratory this year; Dr. Gutierrez will be carrying out structural studies of carbohydrates by NMR and molecular modelling and developing a program in adjuvant chemistry; he moves into a position vacated last year by Dr. Phillips.

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Dr. Terry Henderson has joined the laboratory as an NRC Fellow. His research is directed towards structural studies of the Groups B and C meningococcal polysaccharides in solution and when bound to monoclonal antibodies. This research is being carried out in collaboration with the Dr. Stein's laboratory.

Finally, Dr. Wojciech Stec (Polish Academy of Sciences) is a Guest Worker in our laboratory during his tenure at NIH as a Fogarty Scholar-in-Residence.

In addition to active fundamental research programs (see Research Highlights and Laboratory Bibliography), laboratory personnel have been active participants within the CBER review process. In addition to serving as reviewers on a large number of diverse INDs, all of the senior laboratory personnel are now serving on one or more license committees, with Drs. Bull and Egan additionally serving as PLA chairs. Additionally, laboratory staff serve as reviewers to a number of scientific Journals (J. Amer. Chem. Soc., Biochemistry, J. Biol. Chem., J. Org. Chem., J. Magnetic Reson., etc.), serve as members or reviewers on intramural and extramural NIH Scientific Advisory Panels, and have been invited speakers at many national and international meetings within their scientific specialties as well as to detail FDA regulatory positions.

Research Highlights:

**** NMR Relaxation Theory.** The theory of reversed DEPT and reverse INEPT NMR experiments, useful for the study of protein dynamics, has been developed; the theory also showed ways to minimize errors that could be introduced through multi-exponential relaxation. The theory of dipolar relaxation in the rotating frame was developed to include transverse relaxation in a number of spin systems. (Bull).

**** Internal Molecular Motions and NMR Relaxation.** It has been shown that a generalized Langevin equation cannot exactly reproduce the results of a molecular dynamics simulation of the internal rotation of a methyl groups bound to a macromolecule. A quantum mechanical model, extrapolated to the classical limit, can reproduce the observed results; however, such a model is computationally too involved to be generally useful. Consequently, another model, which shares structural features of the quantum mechanical model but is computationally simpler, is being developed. (Bull & Zhang)

**** Theoretical Studies of HIV-1 Macromolecules.** The effects of solvation on the aspartyl protease from HIV-1 has been studied and results of these studies indicate that the anti-parallel beta-sheet, which acts as a flap covering the enzyme's active site, moves as an independent rigid segment. The N- and C-termini, in contrast, appear to undergo numerous conformational changes. Overall, the structure of the solvated monomer is similar to the crystal structure of the dimer. These studies should prove valuable in future modelling efforts to design protease inhibitors as AIDS drugs. (Venable)

**** Computer modelling of lipid bilayers.** A Molecular Dynamics (MD) simulation of a fluid phase lipid bilayer is the highlight of this year's research from the modelling group. The simulation, which involved nearly 17,000 atoms, required having a suitable initial condition as well as an accurate force field, neither of which were previously available. This simulation will



provide a detailed and accurate atomic level description of the membrane which should be useful for testing theory and complementing experiment as well as enable the modelling group to begin study of the interaction of drugs and proteins with membranes. (Pastor, Hardy, Zhang, and Pastor)

**** Conformational Studies of Nucleic Acids.** The crystal structure of daunomycin with the oligonucleotide, (CGTACG)₂, showed that the drug intercalated between sequential CG base pairs. NMR studies from this laboratory show that the intercalation site is unchanged in aqueous solution and, moreover, show that the drug exchanges on the NMR time scale and provide an average residence time for drug of 10 ms. (Ritter & Byrd).

**** HIV-1 Targeted Drug Therapy.** Various abasic oligonucleoside phosphorothioates were synthesized and evaluated for their ability to inhibit the replication of the virus in a de novo infection assay; the ability to inhibit HIV-1 was roughly a function of the linear charge density. Previous workers have shown that the H-phosphonate of AZT serves as a pro-drug to AZT-5'-monophosphate. The mechanistic basis for this conversion (hydrolysis/phosphorylation vs/ oxidation) is being investigated through the use of an isotopically labelled H-phosphonate. A detailed study of the cellular uptake of a labelled antisense oligonucleotide phosphorothioate has been published.
(Stec, Lesiak, Boal, Egan)

**** Quantitative NOESY Study of carbohydrates: a 1\2 linked disaccharide; Comparison with Molecular Dynamics Simulations.** Extensive 2D NOE measurements have been carried out with the disaccharide, α -L-Rhap-(1\2) α -L-Rhap-(1\OMe) and analyzed using a relaxation matrix based approach. While the NOE studies were in accord with energy minimization calculations carried out using CHARMM, they were not consistent with the results of lengthy molecular or Langevin dynamics trajectories, either in water or in vacuum. Current efforts are now underway to develop a more realistic parameter set for the dynamics calculations so that these may be applied to more complex carbohydrates and glycopeptides. Experimental studies are currently underway in other model systems (Egan, Gutierrez, Hardy)

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G. Barbata, M. Ikura, L. E. Kay, R. W. Pastor, and A. Bax, "Backbone dynamics of calmodulin studied by N-15 relaxation using inverse detected two-dimensional NMR spectroscopy: the central helix is flexible," *Biochemistry*, 31, 5269 (1992).

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CHEMICAL BIOLOGY LABORATORY

Personnel

Blair A. Fraser, Ph.D., Research Chemist, Chief
John C. Hill, Ph.D., Sr. Asst. Scientist, USPHS
Barbara Richardson, Biological Laboratory Technician

The research of the Chemical Biology Laboratory has focused on structural analysis of biologically active peptides and proteins, many of which have undergone some form of post-translational modification.

A variety of post-translational modifications of proteins and peptides have been described in the literature. Examples include glycosylation yielding glycoproteins, acylation yielding lipoproteins and α -N-acetylated cytoplasmic proteins, and phosphorylation yielding oncogenes. Alteration of function due to modification may be dramatic, as in the case of phosphorylation of an oncogene, or insignificant, as in some cases of glycosylation.

Proteolytic processing of precursor proteins remains a dramatic case of post-translational modification. Coupled with other post-translational events, this processing generates a vast array of peptides which serve as important intercellular messengers. One particular aspect, the generation of α -carboxamidated peptides, has been the focus of our research efforts.

The post-translational processing of a pre-pro-hormone occurs in a stepwise fashion. The translation product of a gene for a secreted protein passes into the lumen of the rough endoplasmic reticulum. The protein moves through the Golgi apparatus on its way to secretory vesicles. The pre-pro-protein remains in the membrane-bounded compartment where processing takes place.

Processing at the carboxyl-terminus of a peptide destined to be α -carboxamidated begins with endoproteolysis at a paired basic amino acid site. This proteolysis generates peptides containing basic amino acids, lysine or arginine, at the carboxyl-termini. These basic residues are removed by exopeptidase action to generate the glycine-extended forms of the peptides. The glycine-extended forms serve as substrates for the Peptidyl-glycine α -amidating Monooxygenase (PAM). Products of PAM processing are the α -carboxamidated peptides.

As a general rule, the glycine-extended forms of peptides destined to be α -carboxamidated are biologically inactive. Naturally-occurring α -carboxamidated peptides are biologically active.

We have been using a bovine pituitary tissue extract as a source of new peptides arising from this type of proteolytic processing. We have developed assays that are used to screen column effluents for distinctive post-translationally-processed peptides.

One assay detects carboxyl-terminal glycine which is present on those precursor peptides destined for secretion as α -carboxamidated peptides. The chemical assay for those peptides having carboxyl-terminal glycine is an adaptation of the method of Stark. In our procedure, each peptide fraction is

reacted with an excess of acetic anhydride to form the peptidyl-aminoacyl-mixed anhydrides. Isothiocyanate attack of these mixed anhydrides forms the peptidyl-aminoacyl-isothiocyanates, which cyclize to the 1-peptidyl-2-thiohydantoins. The peptidyl groups are removed by acid hydrolysis. The resulting 5-substituted-2-thiohydantoins are extracted from the reaction mixture and separated by high performance liquid chromatography. The amount of 2-thiohydantoin, formed from glycine, is estimated by comparison with the amount of 5-ethyl-2-thiohydantoin, formed from α -aminobutyric acid added as internal standard.

Assay for those peptides having α -carboxamidated termini is based on oxidative rearrangement of the primary amides into amines followed by derivatization to the 2,4-dithiohydantoins. Peptide fractions containing the peptidyl-aminoacyl-amides are oxidized by an aryl iodine bis-dicarboxylate to peptidyl-gem-diamines. Under basic conditions, the diamines are converted to imines which are attacked by cyanide giving α -aminonitriles. Nucleophilic attack of the α -aminonitriles by carbon disulfide forms thiazolidines, which readily isomerize to 5-substituted-2,4-dithiohydantoins. The dithiohydantoins are extracted from the reaction mixture and then separated by high performance liquid chromatography. The amount of each dithiohydantoin is estimated by comparison with an internal standard.

Synthesis of dithiohydantoins from α -amino acid amides had not been reported previously. Authentic standards were synthesized for each of the naturally occurring amino acids. These standards were purified by crystallization followed by chromatography. Each was identified by plasma desorption mass spectrometry. A few amino acid amides did not form suitable derivatives: tryptophan and tyrosine were oxidatively degraded by the iodoarene; proline formed the thiazolidine; glycine polymerized.

Since no chromatographic methods had been previously published for separation of the 2,4-dithiohydantoins, considerable effort to separate these derivatives resulted in success.

Several peptides have been identified but these arise from proteolysis of myelin basic protein, actin and hemoglobin. Eleven peptides have been purified which were not suitable candidates for sequential Edman degradation because of their solubility in the reagents. Attempts made to sequence small amounts of these peptides resulted in partial amino acid sequences that proved equivocal. Final steps of purification have become very time consuming hindrances in our efforts to structurally analyze novel peptides. Tandem mass spectrometry is a mature analytical method and would be the choice for more rapid identification of new peptide structures. We continue to hopefully anticipate the future availability of such technology.

Over the past year, our efforts have been divided equally between our research effort and regulatory effort. We will continue our research work in identifying and characterizing novel peptides while some laboratory remodeling continues.

LABORATORY OF CELL AND MOLECULAR BIOLOGY SUMMARY

Personnel:

Chief *Philip D. Noguchi, M.D., Captain (PHS)
 *Gerald E. Marti, M.D., Ph.D., Commander (PHS)
 Patricia Carter, Biologist
 Suzanne Sensabaugh, Microbiologist
 Glennelle Washington, Biologist
 Fatima Abassi, M.S., Microbiologist
 *Judith A. Kassis, Ph.D., Senior Staff Fellow
 Robert Metcalfe, M.D., Senior Staff Fellow
 John Bishop, Ph.D., Senior Staff Fellow
 Mary Whiteley, Ph.D., Visiting Scientist
 Jean Hwang, Guest Worker

The Cell and Molecular Biology Laboratory conducts research on the mechanisms of gene expression in normal development and malignancy. The laboratory also operates the Divisional Flow Cytometry/Cell Sorting Facility for independent and collaborative research, as well as for developing potency and specificity assays for the regulation of monoclonal antibody products. Current research efforts include: definition of molecular control mechanisms for the engrailed and other genes in *Drosophila*; characterization of the common chronic lymphocytic leukemia (CLL) phenotype; cell cycle analyses and characterization of ligand-induced cell death.

Dr. Bertin finished his Fogarty appointment in July; Dr. John Bishop joined Dr. Noguchi in January and Dr. Robert Metcalfe joined Dr. Marti's group in July.

Gene Transcriptional Control

Dr. Kassis' group has continued to make significant progress on understanding the mechanisms of gene control in *Drosophila*. Previous work by Kassis and colleagues using evolutionary molecular homology had shown that small (1KB) portions of DNA in the engrailed 1st intron contain enough information to activate a major portion of engrailed gene expression. Remarkably, this same DNA can function as a silencer of an unrelated gene, white, by an apparent transvection mechanism. Two copies of the en fragment are needed to mediate this phenomenon, suggesting that the small piece of DNA may be bound by a DNA binding protein that brings together the two white promoters and extinguishes their effect.

When this same small fragment of DNA is used in a P-element construct, the P element inserts nonrandomly into the *Drosophila* genome near genes that are expressed in a striped pattern, such as en and hairy, as well as near unknown genes. Two of these targeted, unknown genes have been cloned; both have been shown to have classical zinc-finger motifs in their coding regions, which are commonly associated with DNA binding proteins. Other genes are being cloned to identify common sequences that might mediate the specific integration. Taken as a whole, these exciting findings suggest the possibility that minimal fragments of DNA could eventually be used to both enhance and suppress gene expression as a means of gene therapy.

It had been anticipated that comparing *Drosophila engrailed* to an even more divergent species might be able to further define specific areas of gene control by more stringent homology. However, cloning of the mosquito *engrailed* gene showed that, outside of the *engrailed* homeobox, there was little conservation of the *engrailed* protein. This suggests that there is little functional constraint on the *engrailed* protein outside of the homeodomain.

Molecular Medical Genetics

Dr. Marti's efforts on familial CLL have expanded from continued work on the phenotypic characterization of CLL patients to molecular studies of cells of those patients. V region gene structure from five independent CLL lines were studied by PCR amplification and DNA sequencing. All appear to be of the VH3 family, and remarkably, two of the five show a very high DNA sequence homology. This suggests that there may be a subset of CLL that follows a similar immunoglobulin rearrangement, and could provide a means of early detection of CLL in families.

Defining a molecular lesion(s) related to CLL has been complicated by the relative paucity of tools for this disease. In order to develop the methodology, a collaboration with Dr. Elizabeth Raveche has been initiated to exploit the use of NZB mice as a model for human CLL. Using a PCR based linkage analysis, a strategy to identify chromosomal locations for the NZB lesion is being developed. Dr. Marti and Dr. Noguchi have continued to work together on several aspects of flow cytometry, with a major effort placed on standardization of assays for the regulatory control of monoclonal antibodies. As part of this effort, a functional Ethernet network for the flow facility as well as a prototype FDataPlot graphics package have been developed. FDataPlot can display and print a hundred or more one and two-parameter data files on a single sheet of paper. Using such a display has been shown to dramatically decrease the time and effort to spot quality control errors as well as unusual features to flag for further analysis.

Dr. Noguchi has continued his work in cell cycle analyses on a reduced scale because of increased administrative and regulatory responsibilities. Current efforts are focused on identifying by flow analyses those cells undergoing programmed cell death, apoptosis, in response to specific ligands such as IL-4 and tumor antigen-specific monoclonal antibodies.

In addition to their research programs, the senior staff within the Laboratory provide expertise in IND review, preIND and IND meetings and serve and chair license committees, especially in the area of monoclonal antibodies. Dr. Noguchi has been heavily involved in proposing regulations for the control of sperm banks nationwide as well as a member of a PHS Work Group considering the issues of Organ and Tissue Banks in this country. Staff have presented a number of scientific and regulatory papers at various meetings and symposia throughout the year. It is clear that the increasing regulatory responsibility will require skillful prioritization of time; it is hoped that new resources will become available to augment the Laboratory's scientific and regulatory responsibilities.

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MOLECULAR IMMUNOLOGY LABORATORY SUMMARY

Personnel

Suzanne L. Epstein, Ph.D., Chief, Molecular Immunology Laboratory
Steven R. Bauer, Ph.D., Senior Staff Fellow
Ira J. Berkower, M.D., Ph.D., Surgeon, PHS
Frederick W. Miller, M.D., Ph.D., Medical Officer, PHS
James P. Reeves, Ph.D., Research Officer, PHS
Terrance O'Hanlon, Ph.D., Staff Fellow
Lori Love, M.D., Ph.D., Guest Researcher
Sukumar Medda, Ph.D., Senior Staff Fellow
Angelina Mbauba, M.D., Guest Researcher
Julia A. Mispion, M.S., Microbiologist
Rebecca Gurley, M.A., Biologist
Chia-Yun Lo, Biologist
Dano B. Cosenza-Murphy, Biologist
Tin Tran, Guest Worker

(Five summer students)

Research projects ongoing during the past year in the Molecular Immunology Laboratory include work in the following areas: 1) immune responses to HIV, 2) mechanisms of HIV neutralization, 3) antibody variable region repertoire, 4) immunogenicity of different forms of antigens, 5) mechanisms of protective immunity in viral infection, 6) mechanisms of human autoimmune disease, 7) possible relation of silicone exposure to autoimmunity, 8) gene expression in B cell transformation, 9) stages of B cell differentiation, and 10) mechanisms of antigen processing. Several findings can be highlighted:

Dr. Bauer and collaborators have studied B cell tumors arising in transgenic mouse strains he previously derived. The *myc* gene is overexpressed in these mice. Screening for expression of 20 oncogenes revealed abnormal regulation of expression of the p53 tumor suppressor gene or mutation of this gene in about half of the tumors. Dr. Bauer has also used the B lineage tumors arising in these transgenic mice to analyze stages early in the B cell differentiation pathway. He has defined four stages in pro-B and pre-B cell development and determined their order.

Dr. Berkower and coworkers have studied conjugate vaccines in which recombinant HIV gp120 is chemically cross-linked to hepatitis surface antigen, polio, or tetanus as carriers. Conditions and efficiency for cross-linking steps have been analyzed, and studies of immunogenicity have begun. In addition, we have mapped a second major neutralizing epitope of HIV to position 342-511 of the envelope glycoprotein of gp120. Unlike the previously identified neutralizing epitope (V3 loop), this one is conserved among widely divergent HIV isolates and so may be a more suitable target for vaccine development. Other studies used CD4-IgG to neutralize HIV and to reveal features of the mechanism of neutralization. In another project area, antigen processing pathways were studied, with emphasis on the structural requirements for entry and exit from cellular compartments. Previously unidentified signal sequences were shown to alter ability of a protein to traverse certain cellular pathways.

Dr. Epstein and colleagues have analyzed immune responses to two molecular forms of an antigen: recombinant CD4 and native, cell surface CD4. Antibody responses to the two forms preferentially recognized different epitopes, a result with implications for vaccine development. They have completed an analysis of antibody populations induced by anti-idiotypic antibodies in the Ia.7 system, with implications for the nature of this variable region repertoire. In a new research area, they have studied protective immunity to influenza in normal and genetically deficient mice, after vaccination with vaccinia constructs expressing proteins of PR/8 influenza. Mice lacking the gene for β 2-microglobulin, and thus lacking MHC class I antigens and class I restricted cytotoxic T lymphocytes, were protected by HA and NA expressing vaccines just as normal mice were.

Dr. Miller and colleagues have used PCR to study immunogenetic associations of autoimmune diseases called idiopathic inflammatory myopathies. They studied over 200 myositis patients, and have shown an association between certain DR- β and DQ- α genotypes with autoimmune phenotype. Studies of T cell receptor gene expression in autoimmune patients are underway. In addition, they are studying possible relationship of silicone implants and autoimmunity. Clinical, serological, and immunogenetic features have been analyzed in myositis patients with and without silicone implants, and differences detected.

Papers reporting on some of this work have been published or are in press in *Methods in Enzymol.*, *Curr. Top. Microbiol. Immunol.*, *J. Virol.*, *BBRC*, *Arthritis and Rheumatism*, *Medicine*, *Curr. Opin. Rheumatology*, *J. Rheumatol.*, *N. Eng. J. Med.*, *Lancet*, *Clin. Exp. Rheumatol.* In addition, various book chapters have been authored, and several other manuscripts are in preparation.

A major development during the past year was the arrival of Dr. Steven Bauer in October, 1991, and establishment of his lab. In other personnel matters, Dr. Sukumar Medda joined Dr. Berkower's lab in July, 1991, Dr. Terrance O'Hanlon joined Dr. Miller's lab in April, 1992, and Eva Rudikoff will begin work in Dr. Bauer's lab in August, 1992. Neerja Chopra finished his work in Dr. Epstein's lab and departed for medical school. Five summer students are currently working in the various labs.

During the past year, Drs. Berkower and Miller received a total \$115,000 in support from the National Vaccine Program. Renewal applications have been submitted for the next year, and new project proposals have been submitted by Drs. Miller and Epstein. Funding to support student workers was obtained from the Arthritis Foundation, the FAES, and the FDA summer student program.

Molecular Immunology staff reviewed manuscripts for the *J. Immunol.*, *Science*, *PNAS*, *J. Virol.*, *J. Clin. Invest.*, *Cell*, *EMBO Journal*, *Arthritis and Rheumatism*, *Clin. Exp. Immunol.*, and the *JAMA*. Staff were members of a number of clinical and scientific societies. Staff members presented their work at a national review course of the American College of Rheumatology and an NIH conference, Mechanisms of Neoplasia. They also gave lectures at CDER staff college and George Washington School of Medicine.

In regulatory work, Molecular Immunology staff reviewed IND's in the areas of monoclonal antibodies, vaccines, HIV blood test kits, a wide variety of recombinant protein therapeutics, somatic cell therapies, and gene therapies. Dr. Epstein is serving on the licensing committee for a monoclonal antibody used for tumor imaging, and participated in a pre-license inspection. Dr. Berkower is serving on four licensing committees for monoclonal antibodies and blood test kits, including one that licensed a blood test kit this year.

In addition, the staff made specialized contributions. Dr. Bauer participated in an intercenter meeting about regulation of products made in transgenic animals, and is continuing to provide expertise in this area. Dr. Epstein gave seven talks about regulatory concerns in cell and gene therapy, including talks at the NIH "Human Gene Therapy" conference, an NHLBI conference on gene therapy for hemophilia, and "Biologics Update '92" of the Food and Drug Law Institute. In addition, she participated in a committee on policy in the area of somatic cell and gene therapy. Dr. Miller testified on general clinical and epidemiologic aspects of connective tissue diseases to the FDA General and Plastic Surgery Devices Advisory Panel meeting on silicone breast implants in February 1992. He is serving on the CDRH Silicone Breast Implant Clinical Protocol Review Committee and the PHS Silicone Breast Implant Research Task Force. He is also serving as an advisor to CDRH regarding the current ongoing review of other silicone devices. Molecular Immunology staff were regularly consulted by other CBER staff on a wide range of immunological questions related to vaccines, blood products, and monoclonal antibodies.

Molecular Pharmacology Summary

Personnel

Kenneth B. Seamon, Ph.D., Chief
Serge Beaucage, Ph.D., Senior Staff Fellow
Milan Jamrich, Ph.D., Senior Staff Fellow
Malcolm Moos, Jr., M.D., Ph.D., Senior Staff Fellow
Elizabeth McHugh-Sutkowski, Ph.D., Staff Fellow
Marie Louise Dirksen, Ph.D., Biologist
Andra Miller, Ph.D., NRC Fellow
Diane Morris, Chemist
Judy Regan, Chemist
Joan Robbins, Chemist
Marie Krinks, Chemist

The research programs of the Laboratory of Molecular Pharmacology address the general areas of developmental biology, studies of signal transduction, and the synthesis and use of oligonucleotides. These areas of research involve detailed studies which utilize a number of diverse disciplines including chemical synthesis, biochemistry, and molecular biology. The research activities of the Laboratory are augmented by facilities for the solid phase synthesis of oligonucleotides and peptides and also for the purification and microsequencing of proteins and peptides. As such the Laboratory has served as a resource for investigators at FDA and the NIH, as well as other outside investigators. It is intended that the research and resources of the Laboratory will continue to actively provide scientific and regulatory expertise to the Center. In this regard, the research activities directly augment the Center's expertise in addressing a number of regulatory issues. Dr. Moos' and Dr. Jamrich's programs are directly related to the area of developmental biology and the interaction of growth factors in morphogenesis. Dr. Beaucage's research programs are directly related to therapies utilizing antisense oligonucleotides for antiviral therapies. In addition, Dr. Beaucage has served as a resource for evaluating the complex chemistry utilized in synthesizing conjugates of toxins and radionuclides with monoclonal antibodies. Dr. Seamon's and Dr. Moos' expertise in analytical biochemistry has been utilized in a number of issues related to product characterization for growth factors and blood substitutes, genetic stability of continuous cell lines, importance of glycosylation, and the use of synthetic peptides as antigens for in vitro diagnostics.

Synthesis of Modified Oligonucleotides - Dr. Beaucage

Dr. Beaucage has continued his research program in the synthesis and use of modified oligonucleotides. His group developed a new procedure and reagent for the production of oligodeoxyribonucleoside phosphorothioates which was patented and has been used extensively by many investigators throughout the world. Judy Regan in Dr. Beaucage's group developed an improved procedure for the large scale synthesis of the reagent which was published in the Organic Preparations and Procedure International. Dr. Koga, a Fogarty Fellow in Dr. Beaucage's laboratory, synthesized a number of new alternating α,β -oligothymidylates which also served as model antisense molecules. These compounds hybridized tightly to the complementary oligothymidylates and were

more resistant to nuclease activity. Dr. Beaucage is internationally known for his expertise in nucleic acid chemistry. In this regard, he has written to extensive and comprehensive reviews on the synthesis of oligonucleotides by the phosphoramidite approach. These reviews are published in *Tetrahedron*, an extremely prestigious journal read by organic and medicinal chemists.

Regulation of Early Development in *Xenopus* - Dr. Jamrich and Dr. Moos

Dr. Jamrich's research group has been focusing on the identification and characterization of a new family of genes belonging to the Fork head gene family. They have also demonstrated that genes containing a Fork head DNA binding domain are involved in early frog development and play a major role in axis formation as well as in specification of different regions of the neural plate. Current studies are investigating the function of these genes by injection of RNA derived from Fork head genes into developing embryos. This work has been well recognized by the scientific community and Dr. Jamrich has been an invited speaker at many national and international meetings and his work has resulted in two publications and an invited review article.

Dr. Moos has initiated investigations into the role of signal transduction events in morphogenesis. Dr. Moos has extensive experience in studying the regulation of the important signal transducing enzymes, adenylyl cyclase and guanylyl cyclase. In addition, he has collaborated with investigators to identify important factors involved in bone morphogenesis. He is now applying his expertise to identify targets for regulatory genes encoding homeobox domains. Dr. Moos' group is also identifying proteins from the TGF- β family which may play important roles in signal transduction and morphogenesis. Dr. Moos has continued to refine techniques for protein micropurification and sequencing and has applied these to several novel proteins involved with molecular regulation of cell function.

Second Messengers and Cellular Regulation - Dr. Seamon

Dr. Seamon's laboratory has continued studying the interaction of small ligands with membrane proteins. These studies relate to hormone regulation of signal transduction, mechanisms of multidrug resistance and resistance to alkylating agents, and regulation of facilitated glucose transport. In addition, his laboratory has participated in a number of collaborations on the interaction of forskolin with diverse proteins.

Dr. Elizabeth McHugh Sutkowski has continued to investigate the functional differences between the different subtypes of the adenylyl cyclase catalytic subunit. She has demonstrated that there are marked differences between the type 1 and type 2 adenylyl cyclase with regard to their interaction with forskolin and guanine nucleotide regulatory proteins. In addition, she has investigated the potential interactions of forskolin, calmodulin, and other G protein subunits on the type 1 and 2 enzymes. Forskolin binding sites on these proteins are being determined by a combination of methods including covalent affinity labeling and proteolytic digestion experiments. Preliminary data have demonstrated the presence of reactive nucleophilic group at the forskolin binding site on the type 1 and 2 enzymes. Forskolin binding sites on these proteins are being determined by a combination of methods including covalent affinity labeling and proteolytic digestion experiments. Preliminary data have demonstrated the presence of reactive nucleophilic group at the

forskolin binding site on the type 1 and type 2 enzyme. The forskolin binding site also appears to be associated with the N-terminal half of the type 1 enzyme.

Diane Morris has continued to investigate the drug binding site on the P-glycoprotein multidrug transport protein. Diane has identified a region of the protein close to the sixth transmembrane helix which is associated with a drug binding site. This work has resulted in the first definition of a drug binding site on the N-terminal half of the protein. Diane has also participated in studies which have identified P-glycoprotein in the egg membranes of marine organisms. These P-glycoprotein transporters may be involved in reducing the intracellular concentration of toxic materials.

Joan Robbins has continued to develop compounds for the study of protein ligand interactions. Joan has previously developed the synthesis of aminoalkylcarbamates of forskolin which was published in the Journal of Medicinal Chemistry. These compounds have been seminal for studies on adenylyl cyclase, the glucose transporter, and the P-glycoprotein. These compounds have also been utilized for studying the localization of the adenylyl cyclase and the glucose transporter in rat brain using in vitro autoradiography (published in Brain Research). Joan has developed novel derivatives of estramustane which have been used to study the mechanism of action of the anti-cancer effects of estramustane in collaboration with investigators at Fox Chase Cancer Institute.

Regulatory Activities

Personnel in the laboratory continue to contribute to the regulatory activities of the Center. Drs. Moos, Jamrich, Seamon, and Beaucage have participated in the review of IND's related to monoclonal antibodies and other cytokine products. Dr. Beaucage's expertise in organic chemistry has been extremely valuable in evaluating the chemistry utilized for the manufacture of monoclonal antibody conjugates with toxins and radionuclides. Dr. Moos has participated as a member of the licensure committee for ImmuraID anti-CEA imaging monoclonal antibody and also participates on the Hemoglobin Based Oxygen Carriers working group and the Growth Factors working group. Dr. Seamon has participated as a consultant on many licensing committees related to monoclonal antibodies, cytokine products, and in vitro diagnostic test kits. Dr. Seamon has carried out prelicense inspections for many of these products. Staff have also been involved in giving a number of regulatory presentations at meetings.

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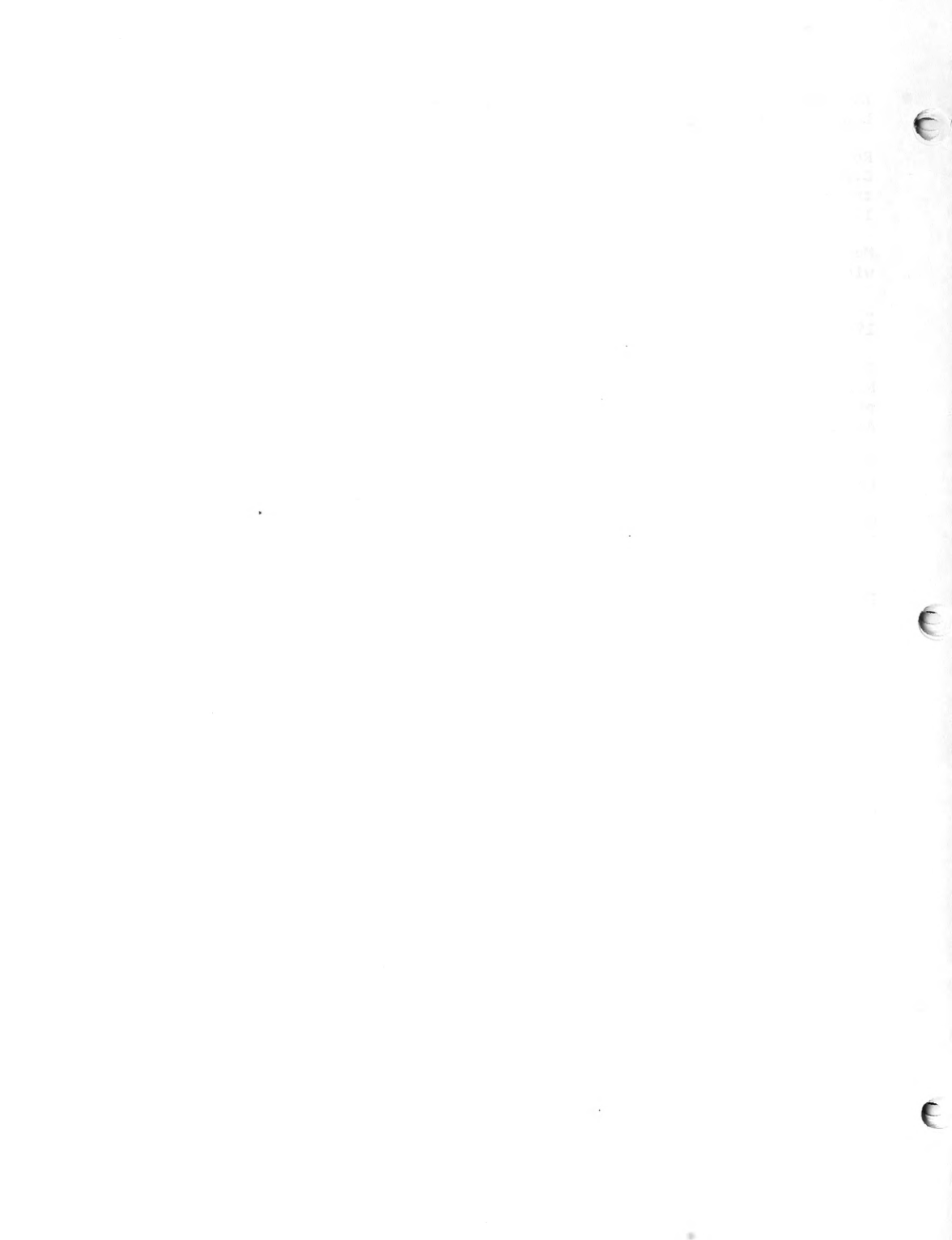
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PERSONNEL IN THE DIVISION

DIVISION OF BIOCHEMISTRY AND BIOPHYSICS 91-92

August 19, 1992

Director: Darrell T. Liu, Ph.D.
 Deputy Director: Philip D. Noguchi, M.D.

Laboratory Chiefs:	Biochemistry Laboratory:	Neil Goldman, Ph.D.
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	Chemical Biology:	Blair A. Fraser, Ph.D.
	Cell & Molec. Biology Laboratory:	Philip D. Noguchi, M.D.
	Molecular Pharmacology Laboratory:	Kenneth Seamon, Ph.D.
	Analytical Chemistry Laboratory:	Joan C. May, Ph.D.
	Molecular Immunology Laboratory:	Suzanne Epstein, Ph.D.

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Fatima Abassi, M.S.	(Microbiologist)
Ira Berkower, M.D., Ph.D.	(Surgeon, PHS)
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Alfred Del Grosso	(Chemist)
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Michelle Elam	(Biological Laboratory Technician, Part-time)
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John Ewell	(Biologist)
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Darrell T. Liu, Ph.D.	(Research Chemist)
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Jila Boal, Ph.D.	(Staff Fellow) (Resigned)
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Rebecca Clayton, Ph.D.	(NRC Fellow) (Termination of Appt.)
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 Jean Hwang
 Wojciech Stec, Ph.D.
 Tin Tran
 Angelina Mbauba, M.D.
 Lori Love, M.D., Ph.D.

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INDIVIDUAL PROJECT REPORTS

ANALYTICAL CHEMISTRY LABORATORY

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-01001-15 LAC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Analysis and characterization of mercurial preservatives in injectables

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Joan C. May, Chief, LAC, DBB, CBER
Nora Eitz, Chemist, LAC, DBB, CBER
Alfred Del Grosso, Chemist, LAC, DBB, CBER
John Moody, Research Chemist, NIST
Diane Bushae, Research Chemist, NIST

COOPERATING UNITS (if any)

National Institute of Standards and Technology

LAB/BRANCH

Laboratory of Analytical Chemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.3

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goals of this project include: (1) the development of methodology for the quantitative analysis of mercury in various biological products (for example, influenza virus vaccine and immune serum globulin) resulting from the use of mercurial preservatives, (2) develop methodology for the quantitative analysis of the thimerosal molecule and any of its degradation products, (3) determine stability of mercury and thimerosal in various products, and (4) determine different mercury species present in various products containing thimerosal such as Immune Serum Globulin, DTP Vaccine, etc. Cold vapor atomic absorption spectrophotometric methodology has been developed to determine the total mercury resulting from mercurial preservatives such as thimerosal, phenylmercuric nitrate and phenylmercuric borate in various injectable biological products. Validation studies for total mercury have been completed for each of the major product types. The cold vapor atomic absorption spectrophotometric procedure used with this sample digestion procedure yields highly accurate and precise results for total mercury in these sample matrices. Thimerosal stability studies are being conducted with a number of different biological products. Thimerosal has been separated from one of its degradation products, thiosalicylic acid, by two reverse phase liquid chromatographic procedures. Liquid chromatography with an amperometric detector, polarography or gas chromatography/mass spectrometry will be explored as methods for the quantitative and/or structural characterization of the thimerosal and its degradation products. Liquid chromatography combined with inductively coupled argon plasma emission spectrometry/mass spectrometry (LC-ICP/MS) has been used to quantitate thimerosal in diluent, a Tetanus Toxoid Adsorbed and an Influenza Virus Vaccine. Work is being done on immune serum globulin by this technique.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

E01-BB-01002-18 LAC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Analysis and characterization of aluminum adjuvant in injectables

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Joan C. May, Chief, LAC, DBB, CBER
 Joseph Progar, Chemist, LAC, DBB, CBER

COOPERATING UNITS (if any)

National Institute of Standards and Technology

LAB/BRANCH

Laboratory of Analytical chemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.6

PROFESSIONAL:

0.6

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this study is to develop atomic absorption spectrophotometric (AAS) methodology for the quantitative analysis of aluminum in various adsorbed toxoids, vaccines and alum precipitated allergenic extracts. The aluminum is present at levels of 0.1 to 0.85 mg/mL. Aluminum phosphate, aluminum potassium sulfate and aluminum hydroxide are the materials used as adsorbents. In addition, information is to be gathered concerning the elemental composition of the toxoids containing the three different aluminum adjuvants and structural characterization of the aluminum adjuvant-antigen complex. Aliquots of samples which contained aluminum suspensions were acid digested with nitric and sulfuric acid and analyzed in the nitrous oxide-acetylene flame of an atomic absorption spectrometer. The 396.2 nm aluminum line was used for analysis. The aluminum content of the National Institute for Standards and Technology (NIST) Standard Reference Material No. 1075a aluminum 2-ethylhexanoate was determined to within 1% of the NIST certificate value by toxoids containing aluminum potassium sulphate and aluminum phosphate were compared with polarographic and inductively coupled argon plasma (ICP) emission spectrometry results. Reproducibility and recovery data for aluminum were determined for a variety of biological products containing aluminum phosphate, aluminum potassium sulphate and aluminum hydroxide adjuvants. In addition, ICP has been used to characterize the aluminum and potassium compositions of the precipitates and supernatant solutions which resulted from centrifugating toxoid suspensions that contained the three different aluminum adjuvants. Mass spectrometry will be explored as a method of determining structural information for the aluminum adjuvant-antigen complexes in the various adsorbed vaccines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-01003-17 LAC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Determination of residual moisture in freeze-dried biological products

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Joan C. May, Chief, LAC, DBB, CBER

Roscoe M. Wheeler, LAC, DBB, CBER

Al Del Grosso, Chemist, LAC, DBB, CBER

Dr. Chiu, DuPont de Nemours and Co., Wilmington DE

COOPERATING UNITS (if any)

DuPont de Nemours and Co.

LAB/BRANCH

Laboratory of Analytical Chemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.8

PROFESSIONAL:

0.8

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (c2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

The goal of this project is to develop new or improved methods for the determination of moisture in biological products. A low residual moisture is necessary for the stability, viability and potency of the freeze-dried biological product. The residual moisture of freeze-dried biological products was first determined by the gravimetric or loss-on-drying method utilizing phosphorus pentoxide and vacuum at room temperature. This method has been optimized. For samples with uncomplicated thermogravimetric analysis (TG) curves, TG results have been shown to correlate with coulometric Karl Fischer results. Karl Fischer and TG moisture results may be different from the gravimetric moisture result for the same freeze-dried product due to the fact that different types of moisture (physically adsorbed or chemically bound moisture) are being measured. The thermogravimetric method has been used to determine the moisture content of Group A and Group C Meningococcal Polysaccharide bulks at levels of 5% to 25% moisture. Thermogravimetric/mass spectrometry (TG/MS) identified the TG transition corresponding to the loss of residual moisture in vaccines that have complex TG curves. Thermogravimetry provides precise heating conditions and weight loss information at specified temperatures, while mass spectrometry identifies volatile compounds evolved during the weight loss process. A new TG/MS interface applicable to this analysis has been developed in our laboratory. The glass tubing interface connects the quartz combustion tube of the TG to the jet separator of the mass spectrometer. This interface allows continuous monitoring of the ion intensities of mass peaks $m/e=18$ (water) and $m/e=44$ (carbon dioxide) for the determination of residual moisture in freeze-dried biological products. Data has been collected clarifying thermograms for both Giant Short Ragweed Allergenic Extracts as well as Limulus Amebocyte Lysate, Haemophilus b Polysaccharide Conjugate Vaccines and other products.

Publication

May JC, Wheeler RM, Etz N, Del Grosso A. Measurement of final container residual moisture in freeze-dried biological products. In: Developments in biological standardization proceedings international symposium on biological product freeze-drying and formulation. Basel: Karger, 1991;153-64.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-01004-12 LAC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Trace metals in injectable biological products

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Joan C. May, Chief, LAC, DBB, CBER

Joseph Progar, Chemist, LAC, DBB, CBER

Ted Rains, NIST

G. N. Biddle, USDA

COOPERATING UNITS (if any)

National Institute of Standards and Technology
U. S. Dept. of Agriculture

LAB/BRANCH

Laboratory of Analytical Chemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.25

OTHER:

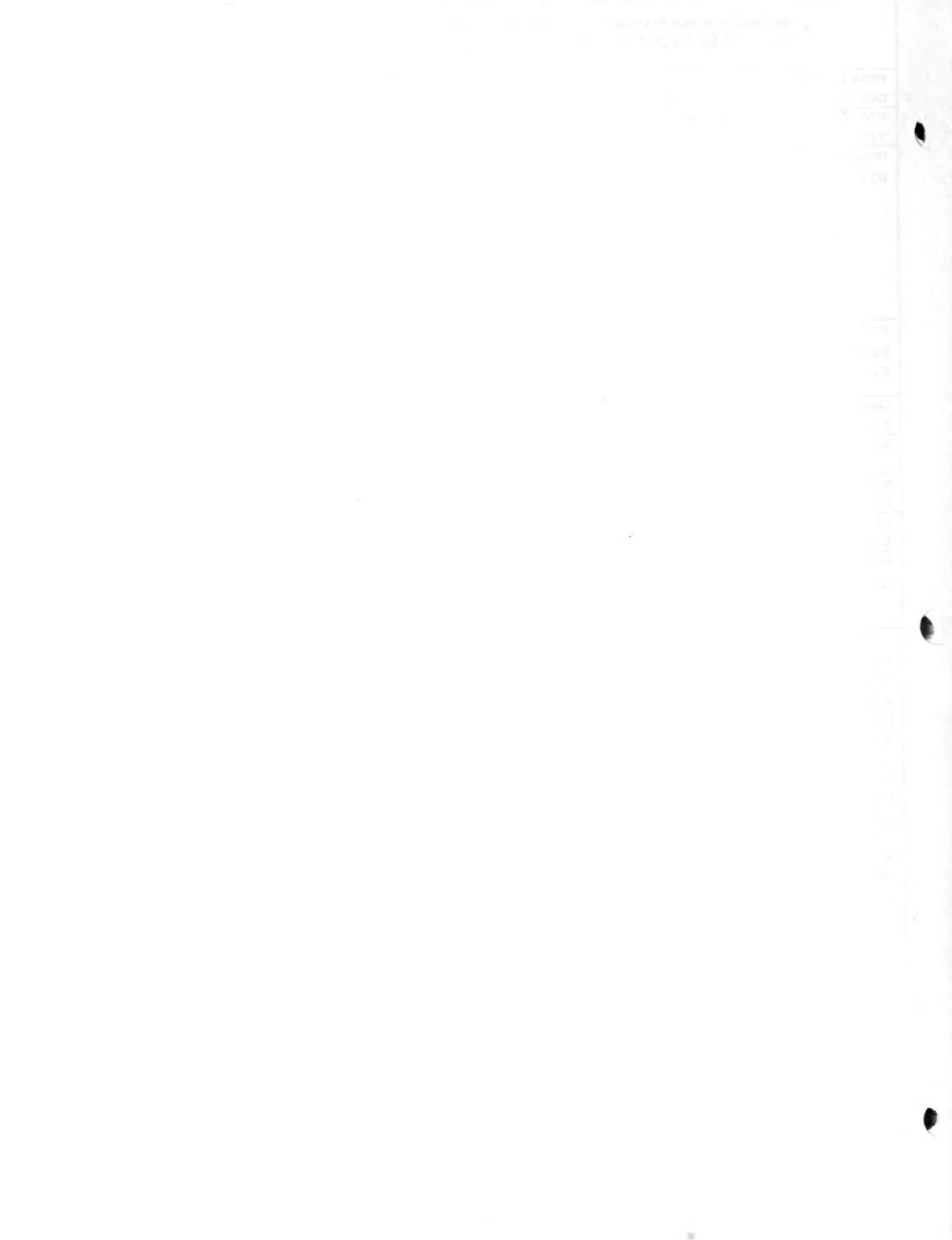
0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Approximately eighty-five samples of injectable biological products regulated by the Center for Biologics Evaluation and Research were surveyed for the presence of eleven elements, namely, aluminum arsenic, barium, cadmium, chromium, lead, mercury, selenium, thallium, and zinc by flame and flameless methods of atomic absorption spectroscopy and flame emission spectroscopy. The majority of the metal concentrations in these products were low or undetectable. The metal levels varied from manufacturer to manufacturer, product to product and lot to lot of the same manufacturer's products. House dust allergenic extract had the highest concentration of arsenic (2.4 ppm), cadmium (0.28 ppm), chromium (0.6 ppm) and lead (1.5 ppm) found in the study. A high zinc concentration (24 ppm) in an immune serum globulin was attributed to the zinc-containing rubber stopper in contact with product. A range of 0.36 to 3.3 ppm albumin was found for seven 25% serum albumin samples from seven manufacturers. Values of 8.2, 17, and 18 ppm albumin were found in one result of an anomaly in this manufacturer's production that has not been repeated to date. Thirty-six samples of source plasma and several samples of anticoagulant and saline associated with source plasma will be analyzed for their aluminum content by electrothermal atomization atomic absorption spectrometry. Source plasma is the starting material for the production of serum albumin. The level of aluminum found in source plasma will indicate the level of aluminum that serum albumin can not be expected to have less than. A reference material prepared at CBER was assayed for albumin by two analytical techniques at the National Institute of Standards and Technology.



May JC, Rains TC, Yu LJ, Etz N. Aluminum content of source plasma and sodium citrate anticoagulant, Vox Sanguinis 1992;62:65-9.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-01005-08 LAC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Determination of Aluminum in Antihemophilic Factor (Human)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Joseph J. Progar, Chemist, LAC, DBB, CBER

Ted Rains, NIST

Joan C. May, Chemist, LAC, DBB, CBER

COOPERATING UNITS (if any)

National Institute of Standards and Technology

LAB/BRANCH

Laboratory of Analytical Chemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.6

PROFESSIONAL:

0.4

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The previous investigation emphasized the use of a non-digestive sample preparation technique prior to the analysis of antihemophilic factor (AHF) preparations for low level aluminum by graphite furnace atomic absorption spectrometry. In order to reduce background absorption, a sometimes severe spectral interference often associated with graphite furnace analysis, sample matrix modification was performed through the introduction of 0.5% sulfuric acid and 0.1% Triton X-100. Further reduction of background absorption was accomplished through modification of the furnace control procedure. Specifically, this involved the addition of an extra drying and a pre-charring step. The graphite furnace technique was applied to a variety of AHF samples including a recent recombinant preparation. Preliminary results show this to be an accurate and precise analytical technique. Data collection will continue in order to further evaluate the precision and accuracy of the method. Future application of the technique will involve investigation into matrix interferences through the use of multipoint standard addition prior to analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-01006-07 LAC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Determination of chlorpheniramine in human blood serum

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Alfred V. Del Grosso, Chemist, LAC, DBB, CBER

COOPERATING UNITS (if any)

Laboratory of Allergenic Products, DBP, CBER

LAB/BRANCH

Laboratory of Analytical Chemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.6

PROFESSIONAL:

0.6

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The determination of chlorpheniramine at low part per billion levels in blood serum is being developed to support a clinical study to be conducted under the direction of Paul Turkeltaub, M.D., Laboratory of Allergenic Products, DBP, CBER. One procedure, that has been evaluated and partially validated, involves extraction, concentration and gas chromatography. Chlorpheniramine is detected by selective ion monitoring mass spectrometry of a characteristic fragment ion at m/e 203. Extraction efficiency and injection volume are normalized by addition of tetradeuterated chlorpheniramine and monitoring of the analogous D4 fragment ion at m/e 207. Development of an alternate method, that would permit the resolution of chlorpheniramine into its enantiomeric components, is in progress. Enantiomeric resolution has not been obtained using gas chromatographic chiral columns. HPLC columns based on alpha-glycoprotein and beta-cyclodextrin stationary phases have provided enantiomeric resolution, but detection of chlorpheniramine at ppb levels has not been accomplished by UV absorbance. Other HPLC detection methods have not been evaluated as providing necessary sensitivity. Work is in progress on a pre-column derivatization method involving reaction with benzylchloroformate, chiral HPLC and fluorescence detection that may provide the necessary sensitivity for detection of chlorpheniramine at serum equilibrium levels while allowing enantiomeric speciation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-BB-01007-07 LAC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Determination of urushiol in allergenic extracts: urushiol stability studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Alfred Del Grosso, Chemist, LAC, DBB, CBER
Paul Turkeltaub, LAP, DBP, CBER

COOPERATING UNITS (if any)

Laboratory of Allergenic Products

LAB/BRANCH

Laboratory of Analytical chemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.6

PROFESSIONAL:

0.6

OTHER:

0

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The active constituents in allergenic extracts of Poison Ivy and other plants of the genus *toxicodendron* have been generically designated as "urushiol", a mixture of penta- or heptadecenyl catechols. Extracts currently available for oral administration or injection are formulated in plant oils, e.g. almond, corn or olive oil. Urushiol compounds may oxidize to orthoquinones which are capable of undergoing Michael-addition reactions with nucleophilic compounds. Olefinic sites on other urushiol molecules may function as nucleophiles, resulting in oligomerization of urushiols present in solution. Objectives of this project are: a) to develop methods for the quantitative analysis of urushiol, including speciation of individual compounds; b) to develop sample preparation techniques to isolate urushiol from plant oil matrices; c) to develop methodologies to determine oxidized and oligomerized urushiol degradation products; d) to correlate urushiol degradation with factors of concentration, solution type and storage conditions. Urushiol samples have been speciated and quantitated by gas chromatography of trimethylsilyl derivatives using a polar stationary phase, OV-225. Validation studies have been performed for this method. A normal phase HPLC method utilizing a silica column is to be investigated as well as a capillary gas chromatographic procedure that would provide better resolution of individual compounds. Procedures for the isolation of urushiol at high (greater than 100 µg/ml) concentrations from plant oils have been validated, development of a solid phase extraction procedure for isolation of urushiol at low (less than 10 µg/ml) concentrations is in progress. Initial studies involving speciation of urushiol oligomers by gel permeation chromatography have been performed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-01008-05 LAC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

High performance metal chelate interaction chromatography

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Alfred Del Grosso, Chemist, LAC, DBB, CBER

James E. Girard, Prof. of Chemistry, The American University

COOPERATING UNITS (if any)

The American University, Dept. of Chemistry

LAB/BRANCH

Laboratory of Analytical Chemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

A diethylenetriamine functional stationary phase, capable of complexing transition metal ions, was developed and evaluated for use in HPLC separations. Synthesis involved evaporation of the trimethoxysilylpropyl-monomer from acetate buffered aqueous solution and derivatization at 200°C and 0.55 torr. Reproducible surface coverages of 3.0 $\mu\text{moles/m}^2$ were obtained on 100-angstrom, 550 m^2/g silica. Analytical columns were loaded with Cu(II), Ni(II) and Zn(II) by frontal elution process; breakthrough volumes corresponded well with metal uptake determined by atomic absorption spectrophotometry. Buffered aqueous-organic mobile phases were used for separations of sulfonamide antimicrobials that included sulfadiazine, sulfa-thiazole, sulfa-merazine and sulfa-pyridine. An empirical retention expression was developed that related chromatographic retention to: a) electrostatic interaction of the acidic sulfonamide function with the cationic stationary phase, b) secondary metal-ligand complexations involving the heterocyclic aromatic substituents of the solutes and c) solubilization of the relatively hydrophobic solutes in the mobile phase. Retentions were inversely proportional to concentrations of organic modifier and buffer and were generally directly proportional to eluent pH. An eluent of pH 7.3 phosphate buffer and 50% methanol was effective in minimizing electrostatic interactions while allowing retention based on metal-ligand interactions. This work was the topic of a Ph.D. dissertation, entitled "Electrostatic and Metal Interaction Chromatography of Sulfonamides" that was defended and accepted in June, 1991. A manuscript based on this study is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-01009-14 LAC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Quantitative determination of phenol, glycerin, formaldehyde, etc. in injectables

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Joan C. May, Chief, LAC, DBB, CBER
Alfred Del Grosso, Chemist, LAC, DBB, CBER

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Analytical Chemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.8

PROFESSIONAL:

0.8

OTHER:

0

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The objectives of this project is to develop methodology and validation data for some constituents of various injectable biological products including: (1) phenol used as a preservative in products such as allergenic extracts and Tuberculin PPD, (2) in products such as allergenic extracts as a preservative and/or stabilizer, (3) pyridine used in the manufacture of certain allergenic extracts, (4) m-cresol used as an anti-microbial preservative in bacterial vaccines, (5) 2-phenoxyethanol used as a preservative in inactivated Polio Virus Vaccine, (6) formaldehyde used as an inactivating agent in Influenza Virus Vaccine, Hepatitis B Vaccine, etc., (7) chloride ion in albumin, and (8) histamine in Histamine, Positive Skin Test Control. Gas chromatographic methods, incorporating internal standards have been developed for phenol, m-cresol and 2-phenoxyethanol. An HPLC method involving pre-column derivatization with p-nitrobenzylhydroxylamine (PNBA) has been developed for the determination of residual formaldehyde in viral vaccines and formaldehyde-modified allergenic extracts. HPLC methods for histamine are being evaluated, one involving pre-column derivatization with o-phthalaldehyde and fluorescence detection, another involving ion-pair chromatography and low wavelength UV detection. Both methods allow the determination of low ppm levels of histamine in the allergenic product, Histamine, Positive, Skin Test Control. A study comparing and validating these two procedures is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-01010-05 LAC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Determination of nitrogen content (protein content) of biological products

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Joan C. May, Chief, LAC, DBB, CBER

Nora Etz, LAC, DBB, CBER

COOPERATING UNITS (if any)

Children's Hospital Allergy Laboratory
Eight U. S. Allergenic Extract Manufacturers

LAB/BRANCH

Laboratory of Analytical Chemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.8

PROFESSIONAL:

0.8

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This study was initiated with the following objectives: (1) to standardize the protein nitrogen unit PNU method for the determination of the concentration of allergenic extracts, (2) to determine the stability of the allergenic extract PNU value throughout the dating period, (3) to determine between laboratory reproducibility for assayed PNI values, and (4) to improve the detection limit for the determination of nitrogen and decrease analysis time. Parameters were optimized for the PNU precipitation procedure for aqueous, freeze-dried, glycerinated and alum precipitated allergenic extracts. The stability study indicated stability for PNU values when the products tested have been stored at a constant 2-8 °C. Although the allergens lose their reactivity with time, the PNU value does not change significantly. It is an estimate of the concentration of a freshly prepared allergenic extract. The collaborative study of the optimized PNU precipitation procedure consisted of the analysis of six samples in duplicate by six laboratories using the CBER Kjeldahl methodology. A chemiluminescence method is being explored as a method for nitrogen determination that is more sensitive than the micro-Kjeldahl method and which can detect about 10 micrograms of protein/mL or 1.6 micrograms of nitrogen/mL. Methodology is being studied to determine the protein content in protein in Typhoid Vaccine, Cholera Vaccine, and other vaccines such as Hepatitis B Vaccine and Hepatitis C100-3 Antigen in which protein measurement by the Lowry method would be subjected to interferences by compounds which are present such as SDS, Tris, EDTA, and thiol reagents (DTT and thimerosal). A study of the above methods as well as an OPA assay is underway.

BIOCHEMISTRY LABORATORY

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-02001-03 LBC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Role of cis-acting Elements in Rubella Virus Replication

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Gregory P. Pogue, Staff Fellow, LBC, DBB, CBER
Hira L. Nakhasi, Research Chemist, LBC, DBB, CBER

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

WORK (Use standard unindented type. Do not exceed the space provided.)

Sequences at the 5' and the 3' end of the rubella virus (RV) genomic RNA can form potentially stable stem-loop (SL) structures and have been implicated to be involved in viral replication. To analyze the function of SL structures in viral translation, we constructed chimeric chloramphenicol acetyl transferase (CAT) RNAs, flanked either by both 5' and 3' SL sequences of the wild type rubella virus genome RNA or several deletion derivatives of the same sequences. The in vitro translational efficiency of chimeric RNAs transcribed by SP6 RNA polymerase was compared in both wheat germ or rabbit reticulocyte lysate translational systems. For in vivo translational studies, the chimeric CAT RNAs were expressed under the control of adenovirus major late promoter in transfected cells and the level of CAT activity was measured. Both in vivo and in vitro translation assays revealed that the presence of 5' and 3' SL sequences of RV RNA, in correct orientation, was necessary for efficient translation of RV/CAT hybrid RNA. The synergism between the 5' and the 3' SL RNA sequences in the translation coincides with their ability to specifically interact with two cellular proteins, 60 kDa Ro/SS-A antigen and calreticulin. The loss of translational capability, by the chimeric RV/CAT RNA, which has altered 5' SL sequence, correlated with its altered Ro/SS-A antigen binding activity. There was a complete inhibition of translation of chimeric CAT RNA in the presence of antibody directed against the Ro/SS-A protein. The role of Ro/SS-a antigen and calreticulin in the translation of RV RNA through their interaction with the SL sequences and its implication in viral replication is currently being studied.

Publications:

) Nakhasi HL, Cao X-A, Rouault TA, Liu T-Y. Specific binding of host cell proteins to the 3' terminal stem-loop structure of rubella virus negative-strand RNA, J Virol 1991;65:5961-7.

Cao X-O, Liu T-Y, Nakhasi HL. The *cis*-acting 3'-element of rubella virus RNA has DNA promoter activity, Gene 1992;114:251-6.

Publication
National
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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-BB-02004-03 LBC
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Purification of Cellular RV RNA-binding Proteins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Nishi K. Singh, Visiting Fellow, LBC, DBB, CBER Darrell T. Liu, Director, DBB, CBER Hira L. Nakhasi, Research Chemist, LBC, DBB, CBER		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Biochemistry		
SECTION		
INSTITUTE AND LOCATION DBB, CBER, FDA, Bethesda, MD 20892		
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p> Rubella virus consists of a single stranded polyadenylated genomic RNA of positive polarity. Sequences at the 5' and 3' end of the genomic RNA can form potentially stable stem-loop (SL) structures and have been implicated in viral replication. Previously, we have demonstrated that cellular proteins specifically interact with both the SL structures and the interaction is necessary for translation. In addition, the binding of a 56 kDa cellular protein to the 3'(+) SL RNA of Rubella virus coincides with the onset of negative-strand RNA synthesis. In this study, we have purified this host protein to homogeneity. The purified protein specifically interacts with the 3'(+) SL RNA with similar affinity as that present in the total cell lysate. Tryptic peptide sequence of the protein was determined and the sequences obtained were compared with the gene bank sequences. The amino acid comparison demonstrated that the 60 kDa purified host protein is calreticulin and is 100% homologous to human calreticulin. Further confirmation of the protein identity was obtained by western blot analysis of 3'(+) SL RNA binding proteins by one or two dimensional PAGE using human calreticulin antibody. The domains of calreticulin responsible for its specific interaction with the 3'(+) SL RNA were identified using tryptic peptides of the protein as competitors in RNA gel shift assay. Currently, studies are underway to define the role of calreticulin interaction in Rubella virus RNA replication. </p>		

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-02006-10 LBC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Molecular mechanism for the regulation of C-reactive protein

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Neil Goldman, Microbiologist, LBC, DBB, CBER
Shi-Peng Li, Visiting Scientist, LBC, DBB, CBER

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

The goal of our project has been the elucidation of the molecular events involved in the regulation of the acute phase response in man. We have concentrated our attention on the prototype acute phase reactant, C-reactive protein (CRP). Our development of an in vitro cell culture system to monitor the induction of acute phase protein synthesis has made it possible not only to determine the extent of de novo CRP synthesis, the type of processing of the protein and the transcriptional level of regulation, but also to determine the types of biological factors responsible for the induction of acute phase reactants. Previously, we found that a protein produced by monocytes induced CRP synthesis. Recently, we found that this protein, referred to as Interleukin-6, is necessary and sufficient to initiate CRP transcription in our cell culture system.

We are also determining the cis-acting elements and the trans-acting factors responsible for the regulatory control of acute phase gene expression. We have isolated the upstream promoter region for the CRP gene and have shown that this region confers inducibility. We have identified both positive and negative regulatory elements, which include 2 distal enhancers and 2 proximal IL-6 responsive elements (6RE) flanking a negative regulatory region. Using mobility shift, methylation interference and immunodepletion assays, we have identified the binding sites and the presence of a number of trans-acting factors: NFIL-6, HNF-1, HNF-3 and several Octamer-like factors. Site-specific mutagenesis of the regions of the 2 proximal 6RE revealed that the upstream element was under inducible positive regulatory control while the downstream element was under inducible negative regulatory control. We are attempting to identify and clone these negative regulatory factors and to determine the modifications which may be altering their binding affinity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-02008-03 LBC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of surface antigen of hepatitis B virus (HBV)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Yuan L. DeVries, Research Chemist, LBC, DBB, CBER

Patty Reeves, Biologist, LBC, DBB, CBER

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

HBV infection is a serious health problem in Southeast Asia and Africa. Due to the increasing numbers of global travelling, HBV infection is no longer a regional disease. Vaccines which are available to the public contain only the small surface antigen of the virus and are proven to be 80 to 90% effective. The presence of the PreS1 and PreS2 in the large antigen might elicit an immune response in non-responders of the vaccines of small antigen. Numerous lines of clinical and experimental evidence have been accumulating which implicate the PreS domains in the infectious cycle of HBV by directly participating in the interaction of virus with host cells.

The objective of the project is to prepare the PreS1 fragment of the surface antigen and the large surface antigen (preS1+preS2 and small antigen) by recombinant DNA technology. The preS1 peptide and the large surface antigen will be used to assess their binding activities to membranes of human hepatocytes and their possible clinical use will be explored.

Lin Y, Liu YX, Cislo T, Mason BL, Yu, M-YW. Expression and characterization of the PreS1 peptide of hepatitis B surface antigen in E. coli, J Med Virol 1991;33:181-7.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-02011-03 LBC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Proteins involved in the defense mechanisms of horse shoe crabs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Yuan DeVries, Research Chemist, LBC, DBB, CBER
 Conceicao Minetti, Visiting Fellow, LBC, DBB, CBER
 Consuelo Dias, Visiting Fellow, LBC, DBB, CBER
 Teresa Liu, Research Chemist, DBB, CBER
 Darrell Liu, Director, DBB, CBER

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Septicemia is a leading cause of morbidity and mortality among hospitalized patients. Despite the use of potent antibiotics and the newly produced monoclonal antibody against LPS, the mortality remains high. In the current studies we have isolated and characterized a number of proteins with agglutination, adhesion and antibacterial activities from the serum and the amebocytes of horseshoe crabs. The purification procedures using ion-exchange and affinity column chromatography resulted in a 55 kDa protein, limunectin, with the ability to bind to Limulus blood cells, to both gram positive and gram negative bacteria and to extracellular matrix. We have also identified a 12 kDa protein with unusual dual functional properties; it binds to endotoxin and also contains protease inhibitory activities. Coagulogen, the substrate for the clot formation, also has agglutinating activities once it is subjected to a limited proteolysis. We are in the process of studying the nature and specificity of such interactions and exploring the lectin properties of these and other components isolated from Limulus blood. Enzymes involved in the clotting cascade are also being investigated.

Publications:

Liu T, Lin Y, Cislo T, Minetti CSA, Baba JMK, Liu, T-Y. Limunectin: A phosphocholine binding protein from Limulus with adhesion promoting properties, J Biol Chem 1991;266:14813-21.

Minetti CSA, Lin Y, Cislo T, Liu T-Y. Purification and characterization of an endotoxin-binding protein with protease inhibitory activity from Limulus amoebocytes, J Biol Chem 1991;266:20773-80.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-02012-04 LBC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Development and Differentiation of Malaria Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Chiang Syin, Staff Fellow, LBC, DBB, CBER

Neil Goldman, Research Microbiologist, LBC, DBB, CBER

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unlined type. Do not exceed the space provided.)

Our objective is to investigate the underlying mechanism in the development and differentiation of malaria parasites, Plasmodium falciparum. The blood-stage parasites of P. falciparum propagate through sexual division (merozoites) and sexual differentiation, which upon intake by mosquitoes will fertilize and preserve its complex life cycle. We have first looked into the function of heat shock proteins (hsps) in parasite development. Hsps are a family of proteins expressed either under stress conditions or constitutively and are well defined among higher eukaryotes. Recent studies of infectious diseases have suggested hsps may play an important role in pathogen development and survival in host. We have identified the genes of two major hsps, hsp60 and hsp90, by screening the genomic DNA and cDNA libraries. Both genes appear to be single-copy genes in the genome. Hsp60 gene is located on chromosome 10. Analysis of their transcripts indicate that the expression of hsp90 is approaching the level of another major hsp, hsp70, which is much higher than that of hsp60. And the expression of hsp90 differs in asexual and sexual stages of parasites, while that of hsp60 remains constant in both stages. We'll next study the signal transduction system in parasites and its role in the development and differentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-02013-04 LBC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protein Chemistry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert Boykins, Biologist, DBB, CBER
Darrell Liu, Director, DBB, CBER

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.1

PROFESSIONAL:

2.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The objectives of our lab projects are:

1. Development of an automated hydrolysis system for proteins and peptides based on the methodology developed in this lab. This methodology has been successfully applied to the analysis of AIDS-related peptides, hepatitis C vaccine, interferons, Pertussis toxin and toxoids as well as erythropoietins.
2. Synthesis of peptides in support of ongoing research and regulatory programs in DBB and CBER.
3. Synthesis of oligonucleotides and protein sequence analysis in support of research and regulatory work within DBB and CBER.
4. Development of analytical method for quantitating methioninesulfoxide in proteins for research and regulatory work within DBB and CBER.

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Publications

Liu T-Y. Glycoprotein pharmaceuticals: scientific and regulatory consideration, and the U.S. Orphan Drug Act, Trends in Biotechnology 1992;10:114-20.

Liu T-Y. Pharmaceutical proteins and peptides: a contemporary perspective. In: Crommelin DJA, Midha KK, eds. Pharmaceutical Sciences. Stuttgart, Hague: Medpharm. Scientific Publishers, 1992;13-1.

Liu T-Y. Deamidation: a source of microheterogeneity in pharmaceutical proteins, Trends in Biotechnology 1992; in press.

Publication

Vol. 1, No. 1, 1954

The Journal of the

Mathematical Society

of the United States

of America

Vol. 1, No. 1, 1954

Page 1

1954

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-02014-02 LBC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Significance of C-Reactive Protein

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Leewin Lin, Staff Fellow, LBC, DBB, CBER
Darrell T. Liu, Director, DBB, CBER

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.2

PROFESSIONAL:

1.2

OTHER:

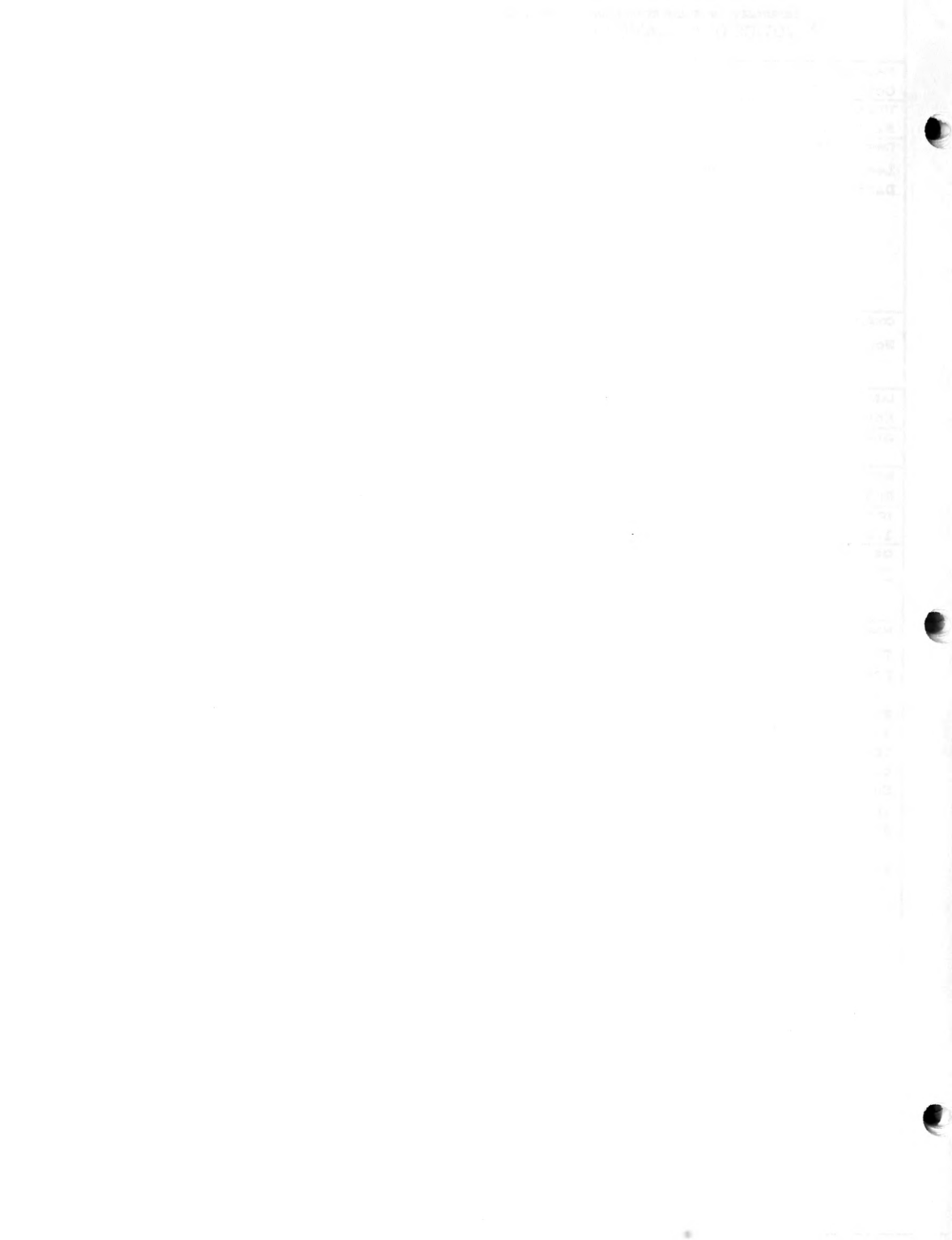
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CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The prototypic acute-phase protein, CRP, is an evolutionarily conserved protein present both in mammals and the invertebrate Limulus polyphemus. To gain further insight into the biological function, mode of production and evolution of CRPs, studies with Xenopus laevis was carried out. CRP was isolated from Xenopus laevis and its amino-terminal sequence determined. Based on this information and the conserved sequence around the putative calcium binding region, and 1.0 kb cDNA clone coding for the entire Xenopus laevis CRP was isolated and sequenced. Subsequently, the CRP gene was also isolated and characterized. RNA blot analysis indicated that the Xenopus CRP is about 1.0 kb in size. Analyses of the protein and nucleotide sequences revealed that the mature CRP is a 222-amino acid protein preceded by a 16-residue signal peptide. The mature human and Xenopus CRPs share 45% identity. Within the sequences of all known CRPs from different species, there are several stretches of highly conserved regions. One of which is the site proposed as the phosphocholine (PC)-binding region. However, the basic amino acids proposed to be important for PC-binding are missing in the Xenopus CRP indicating that either this region is not involved in PC-binding or more likely, that PC-binding does not require positively charged amino acids in this region. Significant differences between human and Xenopus CRP genes were noted. The heatshock consensus sequence and the Interleukin-6 responsible elements found in the 5' end of the human CRP gene are not present in the Xenopus CRP gene. By RNA and Western blot analyses, we have shown that Xenopus CRP gene is not up-regulated during inflammation and is expressed as liver matures but not during early development.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-02016-01 LBC

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cloning and Characterization of Developmentally Regulated Genes from *L. donovani*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Hira L. Nakhasi, Research Chemist, LBC, DBB, CBER

Manju Joshi, Guest Worker, LBC, DBB, CBER

Dennis M. Dwyer, Investigator, LPD, NIAID, NIH

COOPERATING UNITS (if any)

Laboratory of Parasitic Diseases, NIAID, NIH

LAB/BRANCH

Laboratory of Biochemistry

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Differentiation of *Leishmania* from pro- to amastigotes involves both qualitative and quantitative morphological and macromolecular changes. To address this problem, we have developed an *in vitro* culture system for leishmanial growth and differentiation without host cell involvement. We constructed cDNA libraries for both promastigote and amastigote stage of *Leishmania donovani*. By differential cDNA hybridization, we have isolated three cDNA clones encoding mRNAs expressed at different abundance in these stages. We have designated them as P17, A41 and A45. Nucleotide sequence analysis and comparison of deduced amino acid sequence revealed that the P17 clone is homologous to soybean ribosomal protein S11, A41 is homologous to *B. Subtilis* spore germination gene (*gerC*) and A45 has similarity with yeast stress-inducible protein (STI1). The level of RNA for A41 and A45 genes increases several fold when the parasite is grown at 37°C under CO₂ atmosphere whereas at the same time P17 mRNA levels decreased by several fold. Southern blot analysis of all the three genes revealed that they belong to a class of single copy genes. Two out of three developmentally regulated genes, i.e. A41 and A45 are present on chromosome 22, whereas P17 gene is present on chromosome 16. The role of differential expression of three genes with respect to *Leishmanial* development is the subject of future studies.



BIOPHYSICS LABORATORY

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-03002-03 LBP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Theoretical NMR Development I

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Thomas E. Bull, Chemist, LBP, DBB, CBER

Lewis E. Kay, University of Toronto, Toronto, Ontario, Canada

COOPERATING UNITS (if any)

University of Toronto

LAB/BRANCH

Laboratory of Biophysics

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The theory of dipole-dipole relaxation in the rotating frame was developed to include heteronuclear transverse relaxation in AMX, AX-2, and AX-3 spin systems. The theory includes product operator bases as well as bases representing individual lines of the A multiplet. The theory also includes effects of dipole-dipole CSA cross correlation.

Publications

Kay LE, Bull TE. Heteronuclear transverse relaxation in AMX, AX-2, and AX-3 spin systems, J Magn Reson, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-03003-03 LBP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Theoretical NMR Development II

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Lewis E. Kay, LCP, NIDDK, NIH
 Thomas E. Bull, Chemist, LBP, DBB, CBER
 L. K. Nicholson, NIDR, NIH
 C. Griesinger, Univ. of Frankfurt
 H. Schwalbe, University of Frankfurt
 A. Bax, NIDDK, NIH
 D. A. Torchia, NIDR, NIH

COOPERATING UNITS (if any)

NIDDK, NIH
 NIDR, NIH
 University of Frankfurt, Germany

LAB/BRANCH

Laboratory of Biophysics

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.8

PROFESSIONAL:

0.8

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Reverse DEPT and reverse INEPT experiments can be used to measure ^{13}C relaxation with polarization transfer to the much stronger proton signals. In a direct measure of the ^{13}C relaxation, the methyl quartet has the characteristic 1:3:3:1 ratio of peak heights. In straight forward polarization transfer experiments on amino acids in the slow motion regime, however, the experimental ratios of peak heights differed considerably from the above, e.g. -1:11:11:-1 in one case.

The theory of reverse DEPT and reverse INEPT experiments was developed to explain these results and the predictions of those theories were verified experimentally. The theory was then used to show how the 1:3:3:1 ratio could be obtained, thereby providing an accurate substitute for the ^{13}C direct observe experiment. The theory also showed how the experiment could be performed in order to minimize errors due to the multi-exponential relaxation.

Kay LE, Bull TE, Nicholson LK, Griesinger C, Schwalbe H, Bax A, Torchia A. On the measurement of heteronuclear transverse relaxation times in AX-3ds spin systems via polarization transfer techniques, J Magn Reson, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-03004-02 LBP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Theoretical NMR Development III

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Thomas E. Bull, Chemist, LBP, DBB, CBER

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biophysics

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A review article was written on relaxation in the rotating frame. The article includes a historical summary of the subject, a full derivation of the relaxation equations using modern techniques in the field, derivations of the relaxation selection rules, explicit relaxation matrices for a variety of spin systems, and a discussion and analysis of some experimental considerations when using modern spectrometers and techniques.



Bull TE. Relaxation in the rotating frame in liquids, Prog NMR Spec, in press.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-03005-08 LBP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Computer Modeling and Other Theoretical Studies of Biopolymers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard W. Pastor, Chemist, LBP, DBB, CBER
Richard M. Venable, Chemist, LBP, DBB, CBER

COOPERATING UNITS (if any)

DCRT Molecular Modeling Laboratory, NIH
Chemical Physics Laboratory, NIDDK, NIH

LAB/BRANCH

Laboratory of Biophysics

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

12/12

PROFESSIONAL:

6/12

OTHER:

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The purpose of this project is to implement and to further develop the methods of molecular dynamics, stochastic dynamics, Monte Carlo, hydrodynamics and computer graphics to investigate the solution structures of peptides, proteins, nucleic acids and lipid bilayers. The project thus involves questions of both methodology and modeling.

This past year the methodological part of the research principally involved: (i) refinement of the recently developed distribution based Monte Carlo sampling procedure to more accurately describe the conformation of lipid headgroups and all-atom systems; (ii) extensive molecular dynamics (MD) of water, butane and octane to test a variety of force fields, and preliminary simulation of the octane-water interface; (iii) development of a simple hydrodynamic method to calculate diffusion tensors of proteins and DNA by considering only C-alpha carbons and phosphorus atoms, respectively; (iv) Brownian dynamics simulations of a bistable oscillator in order to determine the positional time correlation function and thereby evaluate the importance of memory function corrections to the Optimized Rouse-Zimm Approximation.

Simulations pertaining more to modeling included: (i) MD of a dipalmitoyl phosphatidylcholine (DPPC) lipid bilayer in gel phase where, in agreement with experiment, the system maintained a 30 degree chain tilt and showed disorder in the terminal methyl region; (ii) Langevin dynamics of the disaccharide cellobiose which have enabled the decomposition of motional contributions to the NOE build-up curves; (iii) MD of a DNA 12-mer including water and ions in order to determine the fluid phase, with particular attention being paid to convergence of the deuterium order parameters and the interplay of hydration level and surface area.

CELL AND MOLECULAR BIOLOGY LABORATORY

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-04001-03 LCMB

PERIOD COVERED

October 1, 1991 through July 31, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Molecular biology of chronic lymphocytic leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Pablo Bertin, Fogarty Fellow, LCMB, DBB, CBER
Gerald E. Marti, Senior Investigator, LCMB, DBB, CBER

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cell and Molecular Biology

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

2

PROFESSIONAL:

2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (c2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

We have used a polymerase chain reaction method and nucleotide sequence analysis of the cloned PCR products to identify and characterize the VH, D and JH segments expressed in four EBV immortalized malignant B-cell lines, two of B-CLL origin and two of B-PLL origin. All four cell lines used VH3 family members with greater than 86% homology to reported VH3 sequences. However, only one of the cell lines used a described VH3 family germline gene with little or no somatic mutation. The VH segments utilized by the other three cell lines show less than 95.2% homology to already described VH3 family germline genes. This finding may be explained by somatic mutation and contradicts current accepted ideas about germline VH usage in B-CLL. D germlines used are : DXPl, DIR and D21/9; three use the JH4 gene and all have a variable number of N insertions. In the VH Ig assembly of these chronic B cell malignant cell lines, there seems to be neither preferential VH3 individual member gene usage or D germline gene usage, but there is suggestion of biased JH4 gene usage. Ontogenetically the VH Ig region gene usage in these EBV transformed B-CLL and B-PLL cell lines appear to be more adult-like than fetal.

Bertin PA, Marti GE. Expression of immunoglobulin heavy chain variable gene in B-chronic lymphocytic leukemia and B-prolymphocytic leukemia cell lines. "Restricted" usage of VH family, Proc NY Acad Sci USA, in press.

Publication No.

Book No. 1000

NY 1000

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-04002-03 LCMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Flow Cytometry Network

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Paul Braham, Systex, Inc.

Gerald E. Marti, Senior Investigator, LCMB, DBB, CBER

Philip Noguchi, Chief, LCMB, DBB, CBER

COOPERATING UNITS (if any)

Systex, Inc.

LAB/BRANCH

Laboratory of Cell and Molecular Biology

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

1

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

We have established a network using thin wire ethernet with TCP/IP. An HP98643A ethernet card and Fusion network software (Network Research Corp) was installed on an HP9000 series 300. This network software runs under PWS 3.22 and permits a direct transfer to a FAX Station II GPX running VDS 5.0-1 equipped with a DELQA network module and TCP/IP 5.0 (Wollongoing Software Corp). Once transferred, FCS list mode files are translated to a variety of file formats for selected programs on the FAX and Macintosh. A program called the Laboratory Analysis Package (LAP) which runs on the VAX is used for single parameter, contour and mesh plotting. Hardcopy is available in both Tektronix and PostScript formats. Ethernet cards (Kinetics, Inc.) and network software have been installed on the Macintoshes on which a variety of graphing programs are available. Also, a program has been developed in house called FDAPLOT which currently runs only on the Vax Station. It is highly flexible in laying out any given number of contour and/or single histogram plots. Video display is mouse and window driven; output is in Post Script format. In addition, we have begun to use CAP (Cluster Analysis Program). Two data sets are being explored with this program.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-BB-04005-04 LCMB
PERIOD COVERED October 1, 1991 through September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cell analysis facility: Flow Cytometry Laboratory		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Gerald E. Marti, Senior Investigator, LCMB, DBB, CBER Patricia Carter, Biologist, LCMB, DBB, CBER Fatima Abassi, Microbiologist, LCMB, DBB, CBER Philip Noguchi, Chief, LCMB, DBB, CBER Paul Braham, Systex, Inc.		
COOPERATING UNITS (if any) Systex, Inc.		
LAB/BRANCH Laboratory of Cell and Molecular Biology		
SECTION		
INSTITUTE AND LOCATION DBB, CBER, FDA, Bethesda, MD 20892		
TOTAL STAFF YEARS: 4	PROFESSIONAL: 4	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (c2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The flow cytometry laboratory consists of two FACScans (HP9000) with dual cartridge Bernoulli discs, FACStar Plus (HP9000), a MicroVax II, and a Macintosh II and a Macintosh IICx. All are networked. Several printers including a Tetrionic color printer and LNO3 are available. There are 3 FTE flow cytometer operators capable of doing surface markers and/or cell cycle analysis and cell sorting. A major activity this past year has been the investigation of CD5 and CD20 expression in B chronic lymphocytic leukemia. In addition, we evaluated the serological distribution of the common CLL antigen and its cell cycle relationship. An evaluation of whole blood lysis was completed as part of our studies for individual members of kindred with familial B-CLL. A collaborative review on quantitative flow cytometry was completed. We participated in the NYAS CD5 B-cell meeting and two abstracts were submitted to the International Society of Analytical Cytometry and we attended the Sixth Annual Meeting Clinical Applications of Cytometry. We also participated in M. Potters's meeting on B cell neoplasm. Software programs for data display (LAP, CAP, DCRT) were evaluated and FDAPLOT for the composite display of two parameter contour plots was completed. Our present efforts concern the purification, fractionation, sorting and analysis of blood B lymphocytes and B-CLL lymphocytes to be used in conjunction with PCR methods. Presently we are able to sort sufficient B cells for a PCR analysis of monoclonality. We need to add a separate work station in the flow lab and plan to continue to develop software to meet our needs. We still would like to add digital image analysis to complement our cell analysis. The development of FCM consortium is underway.</p>		

Fleisher TA, Marti GE. Flow cytometric evaluation of human lymphoid cells. In: Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM, Strober W, eds. *Current protocols in immunology*, Vol. 1. New York: John Wiley & Sons, 1991, chapter 7.

Caparosao NE, Whitejouse J, Bertin P, Amos C, Papadopoulos N, Muller J, Whang-Peng J, Tucker MA, Fleisher TA, Marti GE. A 20 year clinical and laboratory study of familial B-chronic lymphocytic leukemia in a single kindred, *Leukemia and Lymphoma* 1991;3:331-42.

Carter PH, Resto-Ruiz S, Washington GC, Ethridge S, Palini A, Vogt R, Waxdal M, Fleisher T, Noguchi P, Marti GE. Whole blood lysis: A flow cytometric analysis of three anticoagulants and five cell preparations, *Cytometry* 1992;13:68-4.

Faguet GB, Agee JF, Marti GE. CD5 and cLLa expression in chronic lymphocytic leukemia (CLL): Demonstration of their relative prevalence and that of other common B-CLL markers, *Leukemia and Lymphoma* 1992;6:335-44.

Faguet GB, Agee JF, Marti GE. Clone emergence and evolution in chronic lymphocytic leukemia: Characterization of clinical, laboratory and immunophenotypic profiles of 25 patients, *Leukemia and Lymphoma*, in press.

Marti GE, Faguet G, Bertin P, Agee J, Washington G, Ruiz S, Carter P, Zenger V, Vogt R, Noguchi P. CD20 and CD5 expression in B-chronic lymphocytic leukemia (B-CLL), *Proc NY Acad Sci USA*, in press.

Faguet GB, Marti GE, Agee JF, Bertin P. CD5 positive and negative B-CLL: Evidence supporting phenotypic heterogeneity in B-chronic lymphocytic leukemia (B-CLL), *Proc NY Acad Sci USA*, in press.

Vogt RF, Marti GE, Schwartz A. Quantitative calibration of fluorochrome intensity for clinical and research applications of leucocyte immunophenotyping by flow cytometry. In: Tyrer HW, ed. *Critical issues in biotechnology and bioengineering*. Norwood NJ: Ablex Publishing Co, in press.

Pepe S, Tortora G, Noguchi PD, Marti GE, Washington GC, Cho-Chung YS. Effects of 8-Cl-AMP and N6-Benzyl-cAMP on cell cycle kinetics of HL-60 leukemia cells, *Cancer Res* 1991;51:6263-67.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

E01-BB-04006-03 LCMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Altering the insertion specificity of a transposable element

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Judith Kassis, Sr. Staff Fellow, LCMB, DBB, CBER
Mary Whiteley, Postdoctoral Fellow, LCMB, DBB, CBER
Suzanne Sensabaugh, Biologist, LCMB, DBB, CBER

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular & Molecular Biology

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.1

PROFESSIONAL:

1.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

P-element vectors are modified transposable elements widely used to make transgenic Drosophila. In general, insertion of P-element vectors is non-random, but exhibits a very broad specificity of target sites. During experiments to identify cis-acting regulatory elements of the Drosophila segmentation gene engrailed (en), we noticed that whenever a particular fragment of en DNA was included within a P-element vector, the specificity for insertion sites was strikingly altered. In particular, P elements containing a small fragment of en regulatory DNA insert at a high frequency near genes expressed in stripes. How does this selective insertion occur? We favor a model whereby a protein(s) bound to the en fragment within the P element brings it to a particular region of the genome via protein-protein or protein-DNA interactions and enhances the probability of insertion within this region. This model presumes that the genes that are "targeted" by the en-containing transposon. Both encode zinc-finger proteins, and thus may act as transcription factors. We are comparing the sequences surrounding these genes in order to determine if common motifs are present which might be recognized by the en-transposon. Our experiments may give us insight into how transposons (or retroviruses) may acquire tissue specificity.

Publications

Kassis JA, Noll E, VanSickle EP, Odenwald W, Perrimon N. Altering the insertional specificity of a *Drosophila* transposable element, *Proc Natl Acad Sci USA* 1992;89:1919-3.

Whitely M, Noguchi P, Sensabaugh SM, Odenwald W, Kassis JA. The *Drosophila* gene *escargot* encodes a zinc finger motif found in snail-related genes, *Mech Dev* 1992;36:117-7.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-04007-03 LCMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A fragment of engrailed DNA can mediate transvection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Judith A. Kassia, Sr. Staff Fellow, LCMB, DBB, CBER
Suzanne M. Sensabaugh, Biologist, LCMB, DBB, CBER
Jean S. Hwang, Summer Student

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.8

PROFESSIONAL:

0.8

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (c2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Transvection is the phenomenon whereby DNA on one chromosome can interact with allelic DNA on the homologous chromosome to control the expression of a gene. We have identified a fragment of engrailed DNA which apparently mediates this phenomenon. This fragment of engrailed DNA can "silence" (i.e. turn off or down) the expression of a linked marker gene. This "silencing" of gene expression occurs only when the engrailed fragment is present in two copies. These two copies can be present either at a similar place on homologous chromosomes or by duplication of the engrailed fragment on the same chromosome. Gene duplication can occur by unequal crossing over or during repair after DNA damage. Our data suggests that gene duplication may in some instances lead to a decrease in gene expression due to the duplication of a "silencing" element. The "silencing" of gene expression could play a role in oncogenesis by the "silencing" of a gene encoding a tumor suppressor gene. We are interested in exploring the mechanism of gene silencing.

PLANT INDUSTRY
BUREAU OF PLANT INDUSTRY
UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

Publication

Kassis JA, VanSickle EP, Sensabaugh SM. A fragment of *engrailed* regulatory DNA can mediate transvection of the *white* gene in *Drosophila*, Genetics 1991;128:571-61.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-04009-04 LCMB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control of gene expression during development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Judith A. Kassis, Senior Staff Fellow, LCMB, DBB, CBER
 Mary Whiteley, Postdoctoral Fellow, LCMB, DBB, CBER
 Rebecca Clayton, MRC Postdoctoral Fellow, LCMB, DBB, CBER (through
 May 12, 1992)
 Suzanne B. Sensabaugh, Biologist

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

PROFESSIONAL:

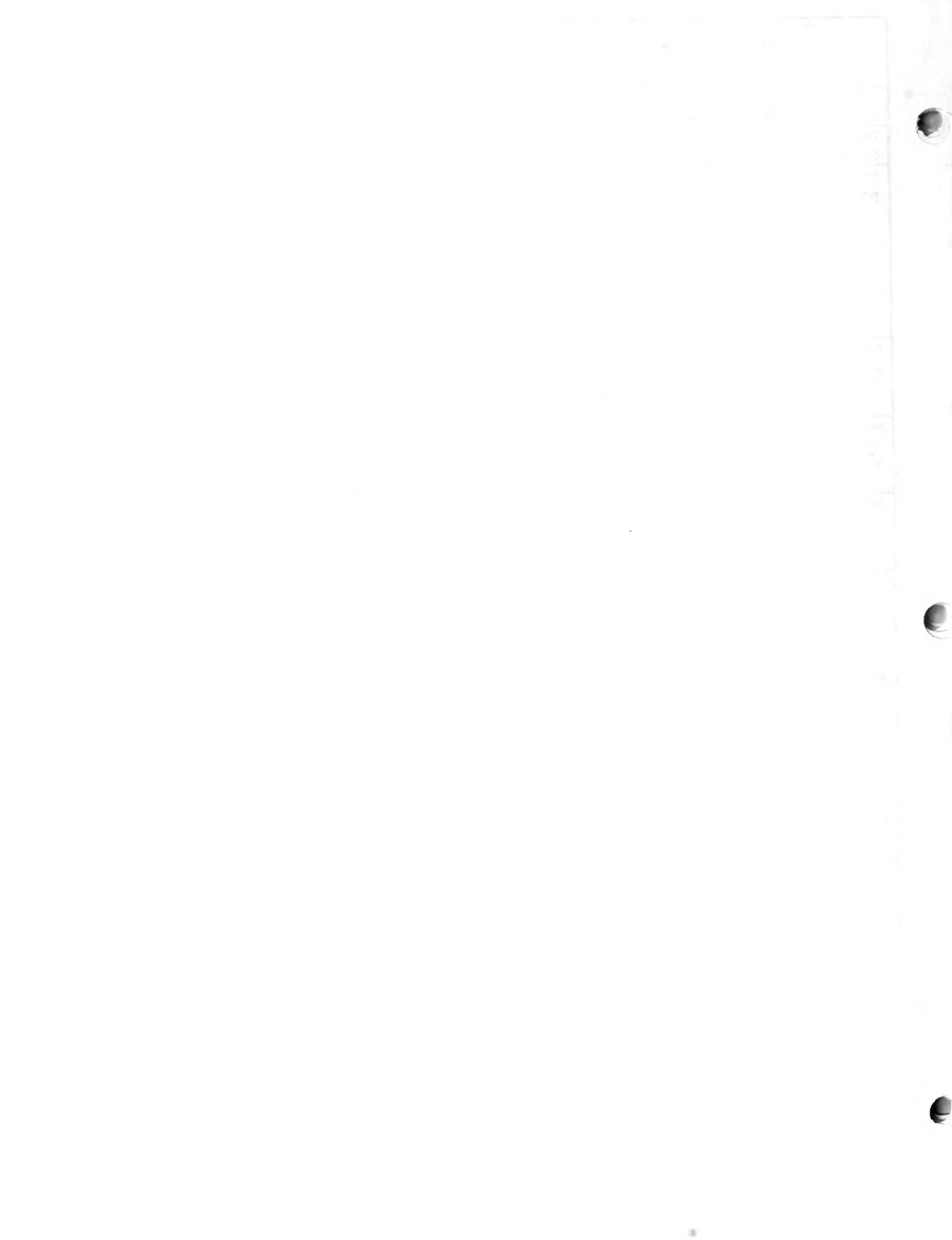
OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The goal of this project is to understand how genes are controlled during development. We are studying the *Drosophila* gene *engrailed* (*en*), which is important for proper segmentation of the embryo and also for formation of the adult. The continually evolving pattern of *en* expression reflects the multiple roles it plays during development. At least 70 kb of regulatory DNA are necessary for regulation of *en* expression. We are interested in how this complex regulatory region evolved and in its conservation throughout evolution. Towards this end, we are cloning the *en* gene from mosquito and studying the conservation of coding and non-coding regions between *Drosophila* and mosquito *en* DNA. We are using functional tests in an attempt to understand the meaning of the conserved sequences. In previous studies, we found that sequences within a 1 kb *en* intron contain the information to give *en* stripes early in development. We are beginning to study this intron in more detail in order to identify proteins which bind to *en* DNA and regulate *en* expression. Through these studies we hope to gain more insight into the complex mechanisms which control development.



CHEMICAL BIOLOGY LABORATORY

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-05001-11 LCB

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Analysis by Mass Spectrometry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B.A. Fraser Chief

LCB, DBB, CBER

Others:

John C. Hill Sr. Asst. Scientist, USPHS

LCB, DBB, CBER

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.4

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Advances in mass spectrometry, due primarily to new desorption techniques, have made it possible to analyze peptides without prior derivatization. Characterization of the covalent structure for a peptide requires more than simply determining the amino acid sequence. Modification of the peptide structure may be due to changes occurring during chemical synthesis of the peptide or may be due to post-translational processing occurring during biosynthesis. Both types of modifications can alter the biological activity of the particular peptide. Identification of these structural changes is necessary for a thorough understanding of the peptide's activity. Generation of structural and functional diversity as a consequence of post-translational modification has continued to be our interest. Prohormone processing is one particular type of post-translational processing that generates structural and functional diversity. Using two assays developed in our laboratory, we have isolated and purified several putative neuropeptide precursors from bovine pituitary and hypothalamus extracts. We continue our search for novel neuropeptides that may arise by post-translational processing of prohormone precursors.

MOLECULAR IMMUNOLOGY LABORATORY

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-06001-05 LMI

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antibody responses to Ia antigens: idiotypes and antibody diversity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Suzanne L. Epstein, Research Chemist, LMI, DBB, CBER
 Julia Mispelon, Microbiologist, MIL, DBB, CBER
 Chia-Yun Lo, Biologist, MIL, DBB, CBER
 Thomas Wood, NCI, NIH

COOPERATING UNITS (if any)

Recombinant DNA Laboratory, Program Resources, Inc. affiliated with National Cancer Institute, Frederick Cancer Research Facility

LAB/BRANCH

Laboratory of Molecular Immunology

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.7

PROFESSIONAL:

0.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Mouse alloantibodies to Ia.7 display a cross-reactive idotype (CRI) that is recognized by xenogeneic anti-idotype, is expressed in all responders of appropriate strains, and is found on all monoclonal anti-Ia.7 antibodies. In addition, both xenogeneic and allogeneic anti-idotypes in this system have striking ability to induce Ia.7-specific responses in mice never exposed to the antigen, providing a model for idiotype vaccination. We have investigated the biological and structural features of the repertoire that form the basis of idiotype sharing in this system. Internal imagery was ruled out as an explanation for the idotype induction. Ia.7-specific antibody populations induced by eight different Ab2 monoclonal antibodies were analyzed for expression of each of the set of idotypes, and the populations found to be distinct in patterns of idotype expression. Mouse anti-idotypic responses failed to recognize the widely shared CRI site, even when sequential immunizations were performed. To examine the structural basis of idotype sharing, light chains of representative idotype-positive mAbs were sequenced under a contract with the laboratories of Drs. Tom Wood and Matthew Gonda, PRI, FCRF, and found to be extremely homologous with each other and with the germ line V κ 21E gene. J1 and J2 were both expressed on CRI+ mAbs. We have proposed a model of overlapping idiotype families to explain the repertoire and biological properties of this response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-BB-06003-07 LMI
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.) Studies of immunity to CD4 and gp120		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Suzanne L. Epstein, Research Chemist, LMI, DBB, CBER James Patton Reeves, Public Health Service, LMI, DBB, CBER Chia-Yun Lo, Biologist, LMI, DBB, CBER Timothy Gregory, Genentech, Inc. Dennis Klinman, DV, CBER Michael Norcross, DCB, CBER		
COOPERATING UNITS (if any) Genentech, Inc. Division of Virology, CBER Division of Cytokine Biology, CBER		
LAB/BRANCH Laboratory of Molecular Immunology		
SECTION		
INSTITUTE AND LOCATION DBB, CBER, FDA, Bethesda, MD 20892		
TOTAL STAFF YEARS: 2.1	PROFESSIONAL: 2.0	OTHER: 0.1
CHECK ALL APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>This project explores the nature of antibody responses to CD4 and to gp120 of HIV. In order to develop appropriate vaccine antigens, responses to different forms of antigens must be better understood. Specific projects:</p> <p>1) Little is known about the comparative immunogenicity of epitopes on recombinant proteins vs native forms present in cells or on organisms. We analyzed antibody responses of mice to human CD4 when presented in recombinant or in cellular form. Mice immunized with rCD4 generate a large response to rCD4, but a lower response to the cell surface form, implying that additional sites are recognized on the recombinant form that are absent in the cellular form. Mice immunized with CD4+ cells had high titers of antibody reactive with whole cells, of which only a small portion was reactive with CD4. Titers on rCD4 are much lower for these mice than in rCD4-immunized mice. Either form of CD4 induced antibodies to the gp120 binding site with comparable efficiency. For another site in domain 3 or 4 of CD4, cellular CD4 induced antibodies more frequently than the recombinant form. Immune response gene differences did not play a detectable role in the anti-CD4 response.</p> <p>2) In collaboration with Dr. Norcross, a series of monoclonal antibodies (mAbs) was derived against recombinant gp120-IIIB of HIV. The goal is to derive mAbs that might distinguish the cell surface form of gp120 from recombinant protein.</p> <p>3) In collaboration with Dr. Klinman, mice were immunized sequentially with rgp120 of different HIV isolates, in an attempt to focus immunity on shared, cross-reactive sites. The mAbs derived did cross-react on multiple isolates in binding assays, but so did mAbs derived by immunization with only one rgp120 isolate. None of the mAbs neutralized.</p>		

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-06004-03 LMI

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

HIV-1 inactivation at low levels of CD4-Ig binding

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ira Berkower, Senior Investigator, LMI, DBB, CBER
Dano Murphy, Senior Technician, LMI, DBB, CBER

COOPERATING UNITS (if any)

Division of Cytokine Biology, CBER (Howard Mostowski)

LAB/BRANCH

Laboratory of Molecular Immunology

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Previous work with recombinant soluble CD4 and CD4-Ig hybrid proteins demonstrated viral inactivation at low levels of CD4-Ig binding. We have extended these studies, using flow cytometry to measure CD4-Ig binding to envelope glycoprotein gp120 on the surface of HIV-1 infected cells. The fluorescent signal increased as a function of CD4-Ig concentration, reaching saturation at 20 nM CD4-Ig, and decreased when subsaturating amounts of CD4-Ig were competed with soluble recombinant gp120, reaching 50% inhibition at <20 nM gp120. Virus survival was measured in a sensitive plaque forming assay over the full range of CD4-Ig binding, between 0 and 99% of maximal binding. Virus survival was essentially 0 whenever CD4-Ig binding was over 20% of saturating. Virus survival reached 37% (1/e) when just 2.7% of available gp120 sites bound CD4-Ig; i.e., when one gp120 molecule out of 36 was bound by CD4-Ig. At each level of average CD4-Ig binding, the fraction of virus surviving equaled the fraction of viruses binding less than this critical value. These results suggest that low level CD4-Ig binding may trigger a conformational change in the virus, converting it to an inactive state. They are consistent with observations that much greater levels of CD4 binding can trigger gp120 shedding from the virus. The recent discovery of fresh isolates, which bind CD4-Ig normally but resist inactivation suggests that these isolates may have lost the triggering mechanism. We are currently studying these isolates for ways to restore triggering.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-06005-03 LMI

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Envelope glycoprotein gp120 conjugate vaccine for HIV-1

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ira Berkower, Senior Investigator, LMI, DBB, CBER
Dano Murphy, Senior Technician, LMI, DBB, CBER

COOPERATING UNITS (If any)

Laboratory of Biophysics, DBB (William Egan)

LAB/BRANCH

Laboratory of Molecular Immunology

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Immunization with envelope glycoprotein gp120 of HIV-1 has met limited success, due to poor immunogenicity in primates and man and due to variability in the virus. Previous work from this lab has identified a site in the carboxyl third of gp120, near the CD4 binding site, as an important target for neutralizing antibodies against HIV-1. Because this site is conserved among diverse HIV-1 isolates, it is an attractive target for vaccines, since antibodies to this site may potentially protect against a broad range of HIV-1 strains. In an attempt to increase the immunogenicity of this site on gp120 without destroying the protein conformation it depends on, we have designed a series of conjugate vaccines in which the gp120 is linked to a stronger immunogen via its carbohydrate sidechains. To this end, we have devised a novel heterobifunctional crosslinker, which links carbohydrate sidechains on the glycoprotein to SH groups on the carrier protein. Model reactions indicate high yield reactions of the crosslinker at both ends under mild conditions. Using protein antigens, coupling efficiency of >90% has been achieved between gp120 and HBsAg, tetanus toxoid, and inactivated poliovirus vaccine. Each conjugate has been used to immunize rabbits, and the resulting antibodies will be tested for the ability to neutralize virus homologous to the envelope used in the vaccine as well as other HIV-1 isolates with divergent envelopes, as a marker of antibodies to the conserved neutralizing site. In this way, we hope to develop conjugates with increased vaccine potency and with the ability to elicit broadly reactive neutralizing antibodies in man.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-06006-03 IMI

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Effect of intracellular protein traffic signals on antigen processing &

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ira Berkower, Senior Investigator, LMI, DBB, CBER
Sumukar Medda, Senior Staff Fellow, LMI, DBB, CBER

COOPERATING UNITS (if any)

Dept. of Transfusion Medicine, Clinical Center, NIH (James Shih)

LAB/BRANCH

Laboratory of Molecular Immunology

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The T cell response to protein antigens depends on antigen processing and presentation by the antigen presenting cell. Proteins are partially degraded into antigenic fragments by one of two processing pathways, and the peptides bind the MHC groove and are transported to the cell surface for presentation to T cells. In this project, we will use normal intracellular traffic signals to manipulate protein entry into each processing pathway and the subsequent transport of processed peptides for MHC binding. For soluble antigens entering the cell by endocytosis, we are studying the effect of adding or deleting a fusion peptide from the amino end. Proteins capable of membrane fusion may enter the cytoplasmic processing pathway directly, without exposure to endosomal proteases, leading to presentation with MHC class I. For endogenously synthesized proteins, we are studying the effect of the endoplasmic reticulum (ER) retention signal KDEL added to or deleted from the carboxyl end. Proteins retained in the ER may be blocked from entering the cytoplasmic processing pathway and may no longer yield peptides for presentation with MHC class I or II. This would establish the site of entry into this pathway as distal to the ER and would also suggest ways to protect foreign proteins from immune recognition. So far, we have prepared plasmid DNA coding for all these protein constructs under control of a T7 RNA polymerase promoter, and we are now testing them for protein expression when co-infected with vaccinia virus expressing the polymerase. If the protein distribution obeys the new signals, we will test each construct for the ability to stimulate T cells specific for the products of one processing pathway or the other.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-06007-02 LMI

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic and environmental factors associated with connective tissue diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Frederick W. Miller, Medical Officer, LMI, DBB, CBER

Rebecca Gurley, Biologist, LMI, DBB, CBER

Terrance P. O'Hanlon, Staff Fellow, LMI, DBB, CBER

Angelina Mbaaya (Interinstitute Genetics Fellow, NIH), Guest Researcher
LMI, CBER

Lori A. Love, CFSAN, FDA

Paul Plotz, Connective Tissue Disease Section, NIAMS, NIH

Ira Targoff, Oklahoma Medical Research Facility, Oklahoma City, OK

COOPERATING UNITS (If any)

CFSAN, FDA

Connective Tissue Disease Section, NIAMS, NIH

Oklahoma Medical Research Facility, Oklahoma City, OK

LAB/BRANCH

Laboratory of Molecular Immunology

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

2

PROFESSIONAL:

2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Individuals who develop diseases in association with antibodies or T lymphocytes which react with self structures are said to have autoimmunity. Autoimmune disorders, many of which are systemic connective tissue diseases, are thought to result from environmental agents acting upon genetically-susceptible hosts with subsequent immune activation and pathology. We are studying a number of these autoimmune diseases to define etiologic factors, to devise more effective therapies, and perhaps ultimately to prevent the occurrence of these diseases. Specific projects include:

1) A study of the human leukocyte antigen (HLA) and T cell receptor (TCR) genes associated with autoantibodies specific to a group of diseases, known as idiopathic inflammatory myopathies, which are characterized by chronic inflammation of muscle or myositis. The laboratory has performed molecular HLA DR, DP, and DQ gene typing by polymerase chain reactions (PCR) using sequence-specific probes and genomic DNA collected from over 200 myositis patients representing all the major clinical and autoantibody groups. There appear to be significant associations of DR-beta and DQ-alpha genes with each of the myositis-specific autoantibodies and with certain clinical groups. This project has also resulted in new PCR technology which has allowed the study of HLA types from biopsy material and human hair when blood has not been available from patients. Studies of TCR gene expression in the periphery and target organs of myositis patients using PCR are also underway.

2) Studies of the epidemiology of myositis are in progress attempting to associate clinical signs and symptoms, geographic location and season of disease-onset, and therapeutic responses in the different clinical and autoantibody subsets. A multicenter study of 377 patients has confirmed that strong associations of the above do exist, and that seasonal and geographic clustering of myositis onset occurs in groups of patients defined by myositis-specific autoantibodies.



Leff RL, Burgess SH, Miller FW, Love LA, Joffe MM, Plotz PH. Distinct clinical and seasonal onsets in myositis patients with anti-Jo-1 and anti-signal recognition particle autoantibodies, *Arthritis Rheum* 1991;34:1391-96.

Love LA, Leff RL, Fraser DD, Targoff IN, Dalakas M, Plotz PH, Miller FW. A new approach to the classification of idiopathic inflammatory myopathies: Myositis-specific autoantibodies define useful homogeneous groups, *Medicine* 1991;70:360-74.

Miller FW. Humoral immunity and immunogenetics in the idiopathic inflammatory myopathies, *Curr Opin Rheumatol* 1991;3:902-10.

Fraser DD, Frank JA, Dalakas M, Miller FW, Hicks JE, Plotz PH. Magnetic resonance imaging in the idiopathic inflammatory myopathies, *J Rheumatol* 1991;18:1693-700.

Miller FW, Leitman SF, Cronin ME, Hicks JE, Leff RL, Wesley R, Fraser DD, Dalakas M, Plotz PH. Controlled trial of plasma exchange and leukapheresis in polymyositis and dermatomyositis, *New Engl J Med* 1992;326:1380-84.

Leff RL, Love LA, Miller FW, Greenberg SJ, Klein EA, Dalakas MC, Plotz PH. Viruses in idiopathic inflammatory myopathies: absence of candidate viral genomes in muscle, *Lancet* 1992;339:1192-95.

Targoff IN, Trieu ED, Plotz PH, Miller FW. Antibodies to glycyl-tRNA synthetase are associated with polymyositis with interstitial lung disease, *Arthritis Rheum* 1992;35:821-30.

Fraser DD, Kong LI, Miller FW. Molecular detection of persistent Borrelia burgdorferi in a man with dermatomyositis, *Clin Exp Rheumatol* 1992; in press.

Tsui FWL, Miller FW. Idiopathic inflammatory myopathies. In: C Bona, et al eds. The molecular pathology of autoimmunity, in press.

Love LA, Miller FW. Understanding the inflammatory myopathies, *Contemp Int Med* 1992; in press.

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37. 2030

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-BB-06008-01 LMI
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mechanisms of protective immunity in mouse influenza		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Suzanne L. Epstein, Research Chemist, LMI, DBB, CBER Julia Mispelon, Microbiologist, LMI, DBB, CBER Chia-Yun Lo, Biologist, LMI, DBB, CBER Brian Murphy, LID, NIAID, NIH Cassie Lawson, LID, NIAID, NIH Kanta Subbarao, LID, NIAID, NIH		
COOPERATING UNITS (if any) Laboratory of Infectious Diseases, NIAID, NIH		
LAB/BRANCH Laboratory of Molecular Immunology		
SECTION		
INSTITUTE AND LOCATION DBB, CBER, FDA, Bethesda, MD 20892		
TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Immunity to viral infections includes both antibody and T cell components. The roles of those components differ under different physiological and genetic circumstances. We have used an <u>in vivo</u> challenge system to reexamine protective immunity to influenza, using as vaccines ten recombinant vaccinia vectors expressing the ten different proteins of influenza. The vaccinia vectors were well tolerated by both normal and beta-2 microglobulin-deficient mice (derived by Drs. Koller and Smithies, obtained from CellGenesys, Inc.). Normal mice were protected against challenge with live influenza virus by H1-VAC and NA-VAC, the classical targets of antibody-mediated protection, but not by vaccinia vectors expressing the eight other flu proteins nor by a mixture of all eight of the latter vectors. Mice genetically deficient in beta-2 microglobulin and therefore expressing no conventional MHC class I antigens were protected in the same cases. Thus, the absence of class I restricted cytotoxic T lymphocytes did not alter observed patterns of protection under these conditions. Genetic control of protective responses and occurrence of cytotoxic T cell immunity are under investigation in mice of several MHC haplotypes.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-06009-01 LMI

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Epidemiology of Silicone-associated Connective Tissue Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Frederick W. Miller, Medical Officer, LMI, DBB, CBER

COOPERATING UNITS (if any)

Dr. Lori A. Love, CFSAN, FDA

Dr. Paul Plotz, Connective Tissue Disease Section, NIAMS/NIH

Dr. Ira Targoff, Oklahoma Medical Research Facility, Oklahoma City, OK

LAB/BRANCH

Laboratory of Molecular Immunology

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

APPROPRIATE BOXES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Although devices containing silicone have been in widespread clinical use for over 25 years, little is known about their safety. Reports of autoimmune and connective tissue disorders following implantation of silicone devices have induced concern among some physicians about a causal relationship, although no adequate data exist to refute or support such claims.

Because investigations of drug-induced autoimmune and connective tissue disorders have documented clinical, serologic and immunogenic differences in these patients compared to those who develop the same "idiopathic diseases" without exposure to the offending drugs, we are studying such differences in myositis patients with and without silicone prostheses. Current data on 13 white women who developed myositis after silicone exposure show significant differences in clinical, serologic, and immunogenetic features compared to 76 sex- and race-matched myositis patients without silicone exposure and compared to normal controls.

These findings are the first to suggest that the presence of certain immune response genes may identify a group of patients at increased risk for the development of connective tissue disease after silicone exposure. Further studies of these and other patients with silicone-associated autoimmune or rheumatic diseases should allow a better understanding of such genetic risk factors, and may allow dissection of pathogenetic mechanisms leading to these diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-06010-01 LMI

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Collaborating genetic mutations in B-cell transformation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Steven R. Bauer, Staff Fellow, LMI, DBB, CBER

Richard M. Scheuermann, Dept. Pathology, Univ. of Texas

COOPERATING UNITS (if any)

Dept. of Pathology, University of Texas, Southwestern Medical Center, Dallas, Texas

LAB/BRANCH

Laboratory of Molecular Immunology

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

We have been studying the multistep process of transformation in B-cell lineage tumors that arise in transgenic mice which overexpress the myc oncogene under transcriptional regulation of mutant or wild type immunoglobulin heavy chain enhancer constructs. In order to identify genes that collaborate in transformation with the inappropriately expressed myc gene, we use a PCR-based assay capable of quantifying expression of 20 candidate oncogenes. Our most interesting observations involve the p53 tumor suppressor gene. In 30% of the B-lineage tumors there is very little if any p53 mRNA. Among those tumors that do express abundant p53 RNA, an additional 20% have point mutations detectable in SSCP assays that examined only two of the eleven p53 exons. An expanded set of SSCP assays covering the remaining p53 exons is currently underway. Thus, a minimum of 50% of the B-lineage tumors have down regulated p53 expression or are expressing mutated versions of the gene. These results strongly suggest that myc gene dysregulation often leads to p53 gene dysregulation and that these two events are common events in the process of B cell tumorigenesis.

Publications

Scheuermann RH, Bauer SR. PCR-based quantification of multiple mRNA species: a method for the analysis of oncogene expression, *Methods Enzymol* 1992; in press.

Scheuermann RH, Bauer SR. Loss of p53 expression in *myc*-induced B lineage tumors, *Curr Top Microbiol Immunol* 1992; in press.

Schumann, H. 1900. Die Bedeutung der
Analyse der menschlichen Sprache.
Schumann, H. 1901. Die Bedeutung der
Analyse der menschlichen Sprache.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE		PROJECT NUMBER
		Z01-BB-06011-01 LMI

PERIOD COVERED
 October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)
 Early stages of B cell differentiation revealed in oncogene transgenic mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
 PI: Steven R. Bauer, Staff Fellow, LMI, DBB, CBER
 Richard H. Scheuermann, Dept. of Pathology, Univ. of Texas

COOPERATING UNITS (if any)
 Richard H. Scheuermann, Dept. of Pathology, University of Texas, Dallas, TX

LAB/BRANCH
 Laboratory of Molecular Immunology

SECTION

INSTITUTE AND LOCATION
 DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)
 We have derived several strains of transgenic mice that use mutant or wild-type versions of the immunoglobulin heavy chain enhancer to over-express the myc oncogene. Normally, the heavy chain enhancer functions only in B cells; however, the mutations relieve constitutive suppression of the enhancer and allow it to activate myc gene expression in non-B cells as well as B cells. The strains with wild-type enhancer constructs invariably succumb to pre-B or mature B cell lymphomas while the transgenic mice with the mutant enhancers get tumors encompassing a wider spectrum of hematopoietic differentiation including pro-B, pre-T, T, and macrophage tumors in addition to pre-B and B cell tumors. Extensive phenotypic and molecular characterization of over 100 early B-lineage tumors has allowed us to characterize 4 stages of pro-B and pre-B cell differentiation and to show their precursor-product relationships along the B cell differentiation pathway.

Bauer SR, Scheuermann RH. Analysis of precursor B-cell differentiation and transformation in *c-myc* oncogene transgenic mice. In: Melchers F, Potter M, eds. Mechanisms in B-cell neoplasia. Basel: Editiones Roche, 1991;345-52.

MOLECULAR PHARMACOLOGY LABORATORY

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

E01-BB-07001-04 LMP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Homeobox Containing Genes During Early *Xenopus* Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Milan A. Jamrich, Senior Staff Fellow, LMP, DBB, CBER

M. L. Dirksen, Chemist, LMP, DBB, CBER

A. Miller, NRC Fellow

COOPERATING UNITS (If any)

Laboratory of Mammalian Genes and Development, NICHD, NIH (K. Mahon)

Laboratory of Pathology, NCI, NIH (S. Mackem)

LAB/BRANCH

Laboratory of Molecular Pharmacology

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

1.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Homeobox containing genes are a family of genes which have been shown in *Drosophila* to control pattern formation during development. These genes encode proteins which bind to DNA in a sequence specific manner. They contain a 180 bp conserved DNA domain called a homeobox. We have isolated several homeobox containing genes which are activated during early *Xenopus* development. We have determined their temporal and spatial expression pattern using Northern blot analysis and *in situ* hybridization. At least some of these genes are expressed during gastrulation, a critical time period of development at which mesodermal and neural induction takes place. Sequence analysis of these genes revealed that some of them are homologous to previously described genes in other species such as mouse and *Drosophila*, others have not yet been described. One of the genes which is homologous to the *Drosophila* Distal-less gene is likely to be involved in limb formation and regeneration. In addition, we have identified a novel gene family which has a similar function as the homeobox genes. They contain a conserved DNA binding motive - the fork head box. Our experiences show that these genes are involved in the regionalization of neural plate as well as mesoderm. The ultimate goal of the project is to understand how the normal development pattern is established and which of the genes play a critical role in this process. Understanding of these processes in frog will give us a better understanding of developmental malformations in humans.

Publications

Dirksen ML, Jamrich M. A novel, activin inducible, blastopore lip specific gene of Xenopus laevis contains a fork head DNA binding domain, Genes Dev 1992;6:599-8.

Taira M, Jamrich M, Good P, Dawid IB. The LIM domain-containing homeobox gene XLIM-1 is expressed specifically in the dorsal lip of Xenopus gastrula embryos. Genes Dev 1992;6:356-6.

Jamrich M, Mahon K. The role of homeobox containing genes in embryonic development. Annu Rep Med Chem 1992; in press.

Publication

Discontinued

Continued

Table No.

Appendix

Journal

Med. Clin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-BB-07002-03 LMP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Forskolin Interactions with Adenylyl Cyclase		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Elizabeth McHugh-Sutkowski, Senior Staff Fellow, LMP, DBB, CBER Joan Robbins, Chemist, LBP, DBB, CBER Kenneth B. Seamon, Chief, LMP, DBB, CBER		
COOPERATING UNITS (if any) Department of Pharmacology, UT Southwestern Medical Center, Dallas TX (W. J. Tang)		
LAB/BRANCH Laboratory of Molecular Pharmacology		
SECTION		
INSTITUTE AND LOCATION DBB, CBER, FDA, Bethesda, MD 20892		
TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0
CHECK ONE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) <p> Forskolin has been demonstrated to interact directly with the adenylyl cyclase catalytic subunit in diverse tissues. However, the ability of forskolin to bind and activate adenylyl cyclase is different depending upon the source of the tissue. Many different types of adenylyl cyclase have recently been cloned. The expression of the different types of adenylyl cyclase in a recombinant Sf9 baculovirus expression system provides an opportunity to study effects of forskolin on specific adenylyl cyclases. Previous work had established that bovine brain adenylyl cyclase binds forskolin with high affinity and has a nucleophilic group at the forskolin binding site. Recent work has established that the binding of forskolin to the Type I adenylyl cyclase is not modulated by the interaction of the Gs protein, in contrast to results with bovine brain. In contrast, the binding of forskolin to the Type II adenylyl cyclase is markedly increased by the interaction with the Gs protein alpha-subunit. Other modulators of adenylyl cyclase including calmodulin, G protein beta-gamma subunits, manganese, and adenosine P site inhibitors do not affect forskolin binding to Type I or Type II adenylyl cyclase. Ligand binding assays have also been developed to study the structure activity relationship for forskolin binding to the expressed adenylyl cyclases. These experiments will provide further information regarding the differences between adenylyl cyclase subtypes and will allow the development of subtype specific derivatives of forskolin. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-07003-04 LMP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synthesis of Forskolin Derivatives with Specificity for Diverse Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.D. Robbins, Chemist, LMP, DBB, CBER
D. Morris, Chemist, LMP, DBB, CBER
K.B. Seamon, Chief, LMP, DBB, CBER

COOPERATING UNITS (if any)

CDER, FDA (N. Appel)
Experimental Diabetes, MNCB, NIADDK, NIH (I. Simpson)
Department of Biochemistry, Cambridge Univ., Cambridge, UK (P. Hendersen)

LAB/BRANCH

Laboratory of Molecular Pharmacology

SECTION

INSTITUTE AND LOCATION

DBB, CBER, FDA, Bethesda, MD

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

SELECT APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

The diterpene forskolin has been demonstrated to affect proteins other than adenylyl cyclase. However, there has been relatively little information regarding the differences in the binding of forskolin at functionally diverse proteins. Analogs of forskolin have been developed which can be used to discriminate between adenylyl cyclase, the facilitated glucose transporter, and the P-glycoprotein multidrug transporter. The hydrogen binding requirements for forskolin interactions at the glucose transporter and the adenylyl cyclase have been determined and are very different. In addition, it has been shown that the affinity of forskolin for the Glut-4 glucose transporter is much higher than for the Glut-1 or Glut-3 transporter. However, the hydrogen bonding characteristics for forskolin binding to the Glut-1, Glut-3, and Glut-4 transporters are not very different. These data will be useful in developing a model for the forskolin binding site at the glucose transporter. Similar experiments are being carried out with adenylyl cyclase subtypes. In addition, experiments are being carried out to determine the structure activity relationships of forskolin for interactions with the P-glycoprotein multidrug transporter.

Whereas the following land is situated in the
 Township of ...
 County of ...
 Province of ...
 and is owned by ...
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Laurenza A, Robbins JD, Seamon KB. An iodinated derivative of forskolin that binds with high affinity to adenylyl cyclase, *Mol Pharmacol* 1991;41:360-8.

Robbins JD, Appel NM, Laurenza A, Simpson I, DeSouza EB, Seamon KB. Differential identification and localization of adenylyl cyclase and glucose transporter in brain using iodinated derivatives of forskolin, *Brain Res* 1992;581:148-52.

Seamon KB. Evaluation of recombinant glycoproteins, editorial in *Glycoconjugate J* 1991; 8:3-5.

Carrow EW, Vujcic LK, Glass WL, Seamon KB, Rastogi SC, Hendry RM, boulos R, Nzila N, Quinnan GV. High prevalence of antibodies to gp120 V3 region principal neutralizing determinant of HIV-1 in sera from Africa and the Americas, *AIDS Research and Human Retroviruses* 1991;7:831-38.

Fratantoni J, Seamon KB. Recombinant erythropoietin: REgulatory issues, In: *Regulatory issues for pharmaceutical products of the new technology*, in press.

Moos M Jr, Seamon KB. Microheterogeneity of biological products. In: *Regulatory issues for pharmaceutical products of the new technology*, in press.

1. Introduction

2. Methodology

3. Results

4. Discussion

5. Conclusion

6. References

7. Appendix

8. Glossary

9. Index

10. Acknowledgements

11. Author's Note

12. Correspondence

13. Contact Information

14. Declaration of Interest

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16. Data Availability

17. Ethics Approval

18. Conflicts of Interest

19. Author Contributions

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42. Correspondence

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44. Declaration of Interest

45. Funding

46. Data Availability

47. Ethics Approval

48. Conflicts of Interest

49. Author Contributions

50. Supplementary Materials

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-07004-03 LMP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Purification and Characterization of Adenylyl Cyclase

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Malcolm Moos, Jr., Senior Staff Fellow, LMP, DBB, CBER
Ken Seamon, Chief, LMP, DBB, CBER

COOPERATING UNITS (if any)

None

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TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

During the past year, the procedures developed previously were used to obtain adenylylcyclase preparations of unprecedented specific activity. Immunoblot analysis of this material using antibodies directed at defined sequences of two different cloned forms of the enzyme indicated that a majority of the enzymatic activity corresponded to a heretofore undescribed isoform.

During the course of these studies, it was discovered that preactivation of the bovine brain membranes used as source material for the enzyme with a non-hydrolyzable GTP analog resulted in enhanced physical association between adenylylcyclase and the beta subunit of the stimulatory GTP binding protein, Gs. Manuscripts describing these findings have been completed and will be submitted shortly, and the project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-07005-03 LMP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

An improved synthesis of oligodeoxyribonucleoside phosphorothioates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. L. Beaucage, Sr. Staff Fellow, MPL, DBB, CBER
J. E. Regan, Research Assistant, MPL, DBB, CBER

COOPERATING UNITS (if any)

None

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0.1

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☐ (a1) Minors
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SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

It is known for some time that antisense phosphorothioate complementary to the messenger RNA of the HIV-1 rev gene inhibited the cytopathic effect of the virus in chronically infected H9 cells. The availability of these oligonucleotide analogues is thus critical for biomedical investigations.

Our laboratory has recently reported the use of 3H-1,2-benzodithiole-3-one 1,1-dioxide as a sulfur-transfer reagent in the synthesis of oligodeoxyribonucleoside phosphorothioates. To promote the accessibility of the sulfur-transfer reagent, Judith E. Regan and the P.I. have scaled up and improved the preparation of the reagent. This procedure has been published in Organic Preparations and Procedures International.

Oligonucleoside phosphorothioates are inherently resistant to nucleases and are therefore difficult to characterize. One approach to the facile characterization of oligonucleoside phosphorothioates would be to convert them to natural oligonucleotides via oxidative desulfurization. Our laboratory has invested considerable efforts in screening various oxidants for the solid-phase conversion of oligonucleoside phosphorothioate triesters to the corresponding phosphotriesters. It has been found that a 0.05 M solution of monoperoxyphthalic acid in glacial acetic acid was ideal for the desulfurization of phosphorothioate oligomers. However, this reagent caused the modification of the cytosine ring. The search for new oxidants for the desulfurization of phosphorothioate oligomer was abandoned.

In addition, the conversion of phosphorothioate oligomers to phosphoramidate oligonucleotides, another class of nuclease-resistant oligonucleotides, has been investigated but has not led to a reagent capable of performing a clean and rapid conversion.

Publications

Regan JB, Phillips LR, Beaucage SL. Large-scale preparation of the sulfur-transfer reagent 3H-1,2-benzodithiol-3 one 1,1-dioxide, Org Prep Proc Int 1992;24:488-2.

Publication

Report 12, 1971
Date of issue

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-07007-02 LMP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of the P-Glycoprotein Multidrug Transporter

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Diane Morris, Chemist, LMP, DBB, CBER
Joan Robbins, Chemist, LMP, DBB, CBER
Kenneth B. Seamon, Supervisory Chemist, LMP, DBB, CBER

COOPERATING UNITS (if any)

Laboratory of Cell Biology, NCI NIH (M. Gottesman)
Oncology and Immunology Research, American Cyanamid Co. (L. Greenberger)

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The P-glycoprotein multidrug transport protein is a membrane glycoprotein which is overexpressed in cell lines and tumors which are resistant to structurally unrelated cytotoxic agents. The exact mechanism of action is unknown, however, the overexpression of the protein results in reduced uptake of cytotoxic drugs. We have been investigating the interaction of forskolin a naturally occurring diterpene, and derivatives of forskolin with the P-glycoprotein. Forskolin photoaffinity agents have been synthesized which are capable of covalent labeling the forskolin binding site. We have demonstrated that the binding site may be identical to that identified by other labeling compounds such as 3H-azidopine and azido derivatives of prazosin. However, the forskolin photoaffinity labels are much more efficient and specific at labeling the P-glycoprotein than other photoaffinity agents. The location of the forskolin binding site on the P-glycoprotein is being determined by digestion of labelled protein and immunoprecipitation with peptide specific antibodies. Forskolin binding appears to be associated with the N-terminal and C-terminal halves of the P-glycoprotein. We have also identified a specific region of the N-terminus which is labeled by forskolin photoaffinity labels. In order to study the binding interactions of forskolin and other agents with the P-glycoprotein, studies have been initiated to develop a high affinity ligand binding assay using iodinated derivatives of forskolin. These studies are aimed at 1) providing a better understanding of the drug binding site; 2) developing forskolin derivatives as potential therapeutic agents; and 3) developing forskolin derivatives as potential in vivo diagnostic agents.

Morris D, Speicher LA, Ruoho A, Tew KD, Seamon KB. Interaction of forskolin with the P-glycoprotein multidrug transporter, *Biochemistry* 1991;30:8371-9.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-07008-02 LMP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

The Synthesis of Oligonucleotides via the Phosphoramidite Approach

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. L. Beaucage, Sr. Staff Fellow, MPL, DBB, CBER

COOPERATING UNITS (if any)

None

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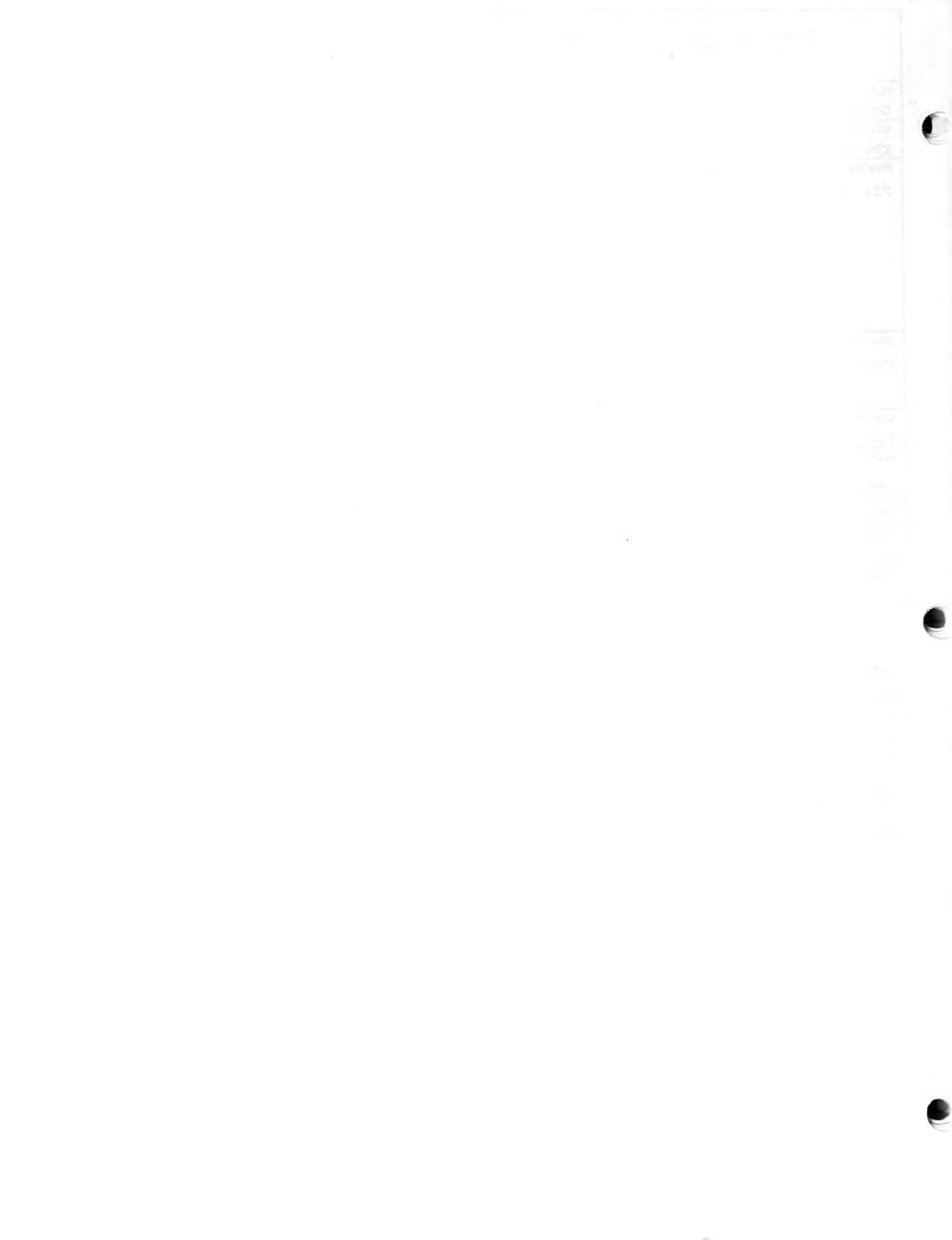
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The P.I. has completed the second of two comprehensive reviews pertaining to the synthesis of oligonucleotides by the phosphoramidite approach and the synthetic application of phosphoramidite derivatives. The first review has appeared in Tetrahedron in March 1992 and has attracted considerable attention. More than 130 reprints have been sent to requestors within two months of the publication date.

The second review is currently being checked for references accuracy and typographical mistakes. The updated manuscript contains more than 600 references and has already been accepted for publication in Tetrahedron. The review will be submitted to the editor by August 1992. It must be noted that these review articles are relevant to the P.I.'s research program and expertise.

The P.I. has recently revised a book chapter about the synthesis of oligonucleotides by the phosphoramidite approach. The editor has informed the P.I. that the book will appear in January 1993.

The editor of Organic Preparations and Procedures International (Dr. J. P. Anselme) has invited the P.I. to write a review about sulfur-transfer reagents. The P.I. may or may not accept this commission depending on the progress of the current research projects underway in the laboratory.



Beaucage SL, Iyer RP. Advances in the synthesis of oligonucleotides by the phosphoramidite approach, *Tetrahedron* 1992;48:2223-11.

Beaucage SL, Iyer RP. Phosphoremidite derivatives and their synthetic applications, *Tetrahedron* 1993;49:0000-00.

Beaucage SL. The synthesis of oligodeoxyribonucleotides by the phosphoramidite approach. In: Agrawal S, ed. *Synthetic chemistry of oligonucleotides and analogs*. New Jersey: Humana Press, 1993;0000-00.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-07009-02 LMP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Alternating α , β -oligothymidylates as model antisense molecules

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. L. Beaucage, Sr. Staff Fellow, MPL, DBB, CBER
M. Koga, Fogarty Postdoctoral Fellow, MPL, DBB, CBER

COOPERATING UNITS (if any)

None

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TOTAL STAFF YEARS:

0.3

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

Alternating α , β -oligothymidylates with alternating (3'→3')- and (5'→5')-internucleotidic phosphodiester linkages have been synthesized to evaluate the stability of these oligonucleotide analogues to nucleases and their hybridization abilities. It was found that these oligomers hybridized to their complementary β -oligodeoxyriboadenylate as tightly as phosphorothioate oligomers. The α , β -oligothymidylates were also more resistant to the nucleolytic activity of S1 nuclease than that of the phosphorothioate and β -oligothymidylates.

During the last months of his tenure in our laboratory Dr. Koga has synthesized the four different α -deoxyribonucleoside phosphoramidites in an attempt to prepare an α , β -oligonucleotide complementary to the mRNA encoded by the rev gene of HIV-1. The preparation of α -deoxyguanosine and its phosphoramidite has been particularly difficult. Since the departure of Dr. Koga in January 1992, this project has not progressed. A replacement for Dr. Koga has not, as yet, been approved. More data are required to complete the study of this interesting class of oligonucleotides achiral at phosphorus. Specifically, it would be worth gathering various physico-chemical properties (thermal denaturation and CD analysis) and extended nucleolytic resistance data before applying these oligonucleotide analogues to antisense experiments.

Koga M, Moore MF, Beaucage SL. Alternating α,β -oligothymidylates with alternating (3'→3')- and (5'→5')-internucleotidic phosphodiester linkages as models for antisense oligodeoxyribonucleotides, J Org Chem 1991;56:3737-59.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-07010-02 LMP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Cellular Uptake of Oligonucleotide Analogues

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. L. Beaucage, Sr. Staff Fellow, LMP, DBB, CBER
M. Koga, Fogarty Postdoctoral Fellow, LMP, DBB, CBER
J. B. Regan, Research Assistant, LMP, DBB, CBER

COOPERATING UNITS (if any)

None

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TOTAL STAFF YEARS:

0.7

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0.7

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0.5

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☐ (a1) Minors
☐ (c1) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Improving the cellular uptake of oligonucleotide analogues represents a critical issue with respect to the application of these biomolecules in the targeting of mRNAs or in the formation of triplex structures with genomic DNA as a means to control gene expression.

Our approach entails the systemic tailing of oligonucleotides with monomeric polyethylene glycols at either the 3'-end, 5'-end or both ends. Dr. Koga and Judith B. Regan have recently coupled various polyethylene glycol macromolecules (M.W. 2,000-10,000) onto a controlled-pore glass support (CPG) and have synthesized oligonucleotides from these derivatized supports. This approach led to the facile preparation of oligonucleotide-polyethylene glycol conjugates. All attempts to couple polyethylene glycol macromolecules at the 5'-end of oligonucleotides anchored to CPG, failed. The above conjugates had characteristic mobility on polyacrylamide gel electrophoresis and have not affected hybridization with unmodified complementary oligonucleotides. The T_m s of the modified duplexes were similar to that of the native DNA duplexes. To facilitate the monitoring of the cellular uptake of such conjugates, Judith B. Regan has synthesized a fluorescent marker that will be inserted between the polyethylene glycol and the oligonucleotidic moieties of the conjugates.

In addition to be applied to the study of cellular uptake, PEG-oligonucleotide conjugates will also be hybridized to large (500 bp) complementary mRNAs to evaluate *in vitro*, the effect created by the steric hindrance of the polyethylene glycol tail on the translation of these mRNAs relative to proper controls.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-07011-02 LMP

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October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Primary Structure Analysis of Regulatory Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Malcolm Moos, Jr., Senior Staff Fellow, LMP, DBB, CBER

COOPERATING UNITS (if any)

None

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Since the last report, continued improvements in analytical technology have extended the lower practical limit for internal amino acid sequence analysis of proteins separated by gel electrophoresis to the vicinity of 50 pmol. This has allowed analysis of several novel proteins and has facilitated analysis of proteins previously under study.

Sufficient sequence information was obtained for the cyclic GMP-inhibited phosphodiesterase described in the last report to allow cloning and expression. Approximately 300 residues of unique sequence have been obtained for a novel neuronal calmodulin binding protein. Though sufficient sequence has been obtained to account for over 60% of the protein's primary structure, no significant homology with other mammalian proteins has been detected. A protein demonstrating UV irradiation-induced DNA binding activity has been sequenced, allowing isolation of the full-length cDNA.

During studies related to purification of adenylyl cyclase (Z01-BB-07004-03), several proteins binding to forskolin affinity supports were observed. Three of these have been identified as the cytoskeletal proteins tubulin, actin, and clathrin.

Several proteins binding the putative second messenger Ap5A were also identified by sequence analysis as various glycolytic enzymes, indicating the possibility of a novel level of regulation of these processes.

The general utility of these techniques has also been used to support efforts within CBER to identify the particular components within allergenic materials that are responsible for their untoward effects with the eventual goal of allowing better standardization of allergenic products. Primary sequence from apple and wheat antigens that react with sera from allergic patients has identified them as previously undescribed proteins.

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Publications

Moos M Jr. Determination of internal amino acid sequence from electrophoretically proteins. In: Current protocols in immunology. New York: Greene/Wiley-Interscience, 1992.

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Meacci E, Taira M, Moos M Jr, Smith CJ, Movsesian M, Degerman E, Belfrage P, Manganiello V. Proc Natl Acad Sci USA 1992;89:3721-25.

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Greco NJ, Yamamoto N, Jackson BW, Tandom NN, Moos M Jr, Jamieson GA. Identification of a nucleotide binding site on glycoprotein IIb: relationship to ADP-induced activation, J Biol Chem 1991;266:13627-33.

Manch-Citron J, Allen J, Moos M Jr, London J. The gene encoding a *Prevotella loescheli* lectinlike adhesin contains an interrupted sequence which causes a frameshift, J Bacteriol 1992; in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-BB-07012-01 LMP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Signal Transduction Events in Morphogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Malcolm M. Moos, Jr., Senior Staff Fellow, LMP, DBB, CBER

Milan Jamrich, Senior Staff Fellow, LMP, DBB, CBER

COOPERATING UNITS (if any)

None

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TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.75

OTHER:

0.25

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- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The events of pattern formation and tissue differentiation are now thought to be controlled in large part by the actions and interactions of peptide growth factors, their receptors, and transcription factors which may either be acted upon as a result of growth factor release or which may direct the synthesis of these factors and their receptors. Unfortunately, the pathways connecting these components remain unclear. Since many products currently under review by CBER belong to the category of growth regulatory molecules, it is important to understand their actions. We have therefore begun attempts to identify targets for important regulatory genes containing homeobox domains. At present, we have expressed one of these proteins for preparation of antisera and are in the process of doing the same for several other genes. These will be used for immunoaffinity purification of the DNA to which the regulatory proteins are bound; cloning and sequencing of this DNA will allow identification of the gene. We have also identified potential homeobox genes in zebrafish embryos, which possess several useful characteristics that may complement the work in amphibians.

In addition, candidates for members of the TGF-beta receptor family have been identified with PCR technology. In future studies, we hope to exploit DNA-protein interactions to identify trans acting factors which regulate these and other molecules involved in signal transduction.

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